Tree Planters' Notes

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***Tree Planters' Notes* (TPN) is dedicated to technology transfer and publication of information relating to nursery production and outplanting of trees and shrubs for reforestation, restoration, and conservation.**

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Editor: Diane L. Haase

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Fall 2013

Dear TPN Reader

This issue contains two more articles in *Tree Planters' Notes* ongoing series to highlight tree planting activities in every State (Iowa, p. 4, and Idaho, p. 19). It's quite interesting to read the differences and similarities in historical and current practices with regard to reforestation and restoration across the country. A common theme is the mass destruction of forests that occurred in the 19th and early 20th centuries followed by establishment of State and Federal policies and programs to address the resulting soil erosion, depletion of forest resources, and challenges to establish new forests. Another commonality is the many current and future issues associated with changing climates and environments; urban encroachment; and invasive pathogens, insects, and weeds. Nonetheless, there is also much diversity among States in their native tree species, natural resource management approaches, and tree planting practices.

This issue also includes new and useful research applicable to tree planting. French and Meilan (p. 27) studied the effects of temperature, photoperiod, and seed scarification on germination of nine ash species. Starkey and Enebak (p. 35) compared root quality of seedlings lifted with different nursery seedling lifters operated at different speeds. Baldet and Colas (p. 43) describe a quick and simple technique for drying seeds and pollen. deGraan and colleagues (p. 50) developed a seed stratification protocol for lab germination tests designed to correlate with operational stratification methods and improve sowing calculations. Jetton and colleagues (p. 59) describe a cooperative genetic resource conservation program for eastern hemlock and Carolina hemlock, two species that are threatened by the hemlock wooly adelgid.

In addition, this issue contains a report on forest nursery seedling production in the United States during the previous fiscal year (p. 72) that provides quantitative estimates of hardwood and conifer as well as bareroot and container seedlings produced and planted in each State and each region.

I'm looking for more articles to fill the 2014 issues of *Tree Planters' Notes*. Please consider submitting your paper! Guidelines for authors can be found at the end of this issue as well as online at http://www.rngr.net/publications/tpn.

Kind Regards,

Diane L. Haase

Contents


SEE PAGE 37


SEE PAGE 67

Forestry and Tree Planting in Iowa

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Iowa's Forest History

Trees provide multiple benefits for wildlife, shade, windbreaks, beauty, recreation, clean air, clean water, and wood products to everyone living in Iowa. After it was discovered that Iowa's soils were extremely productive, the transformation of native vegetation resulted in one of the most altered landscapes in the world. Early maps (1832 to 1850) show about 6.7 million ac (2.7 million ha) or 19 percent of Iowa was covered with timber, out of the total of 35.5 million ac (14.4 million ha) in the State (figure 1). Over time, the forest habitat has been fragmented and dramatically reduced in size. Iowa has never returned to growing as many acres of forest as it had 380 years ago (figure 2). Historic forest maps provide a footprint to begin prioritizing areas to improve the quality, quantity, and connectivity of existing forests today.

During the time of early statehood, Iowa forests produced many important commercial timber species that are common in the central hardwood region. These important tree species included black walnut (*Juglans nigra* L.), 11 species of oak (*Quercus* spp.), basswood (*Tilia americana* L.), American elm (*Ulmus americana* L.), red elm (*U. rubra* Muhl.), several species of hickory (*Carya* spp.), white ash (*Fraxinus americana* L.),

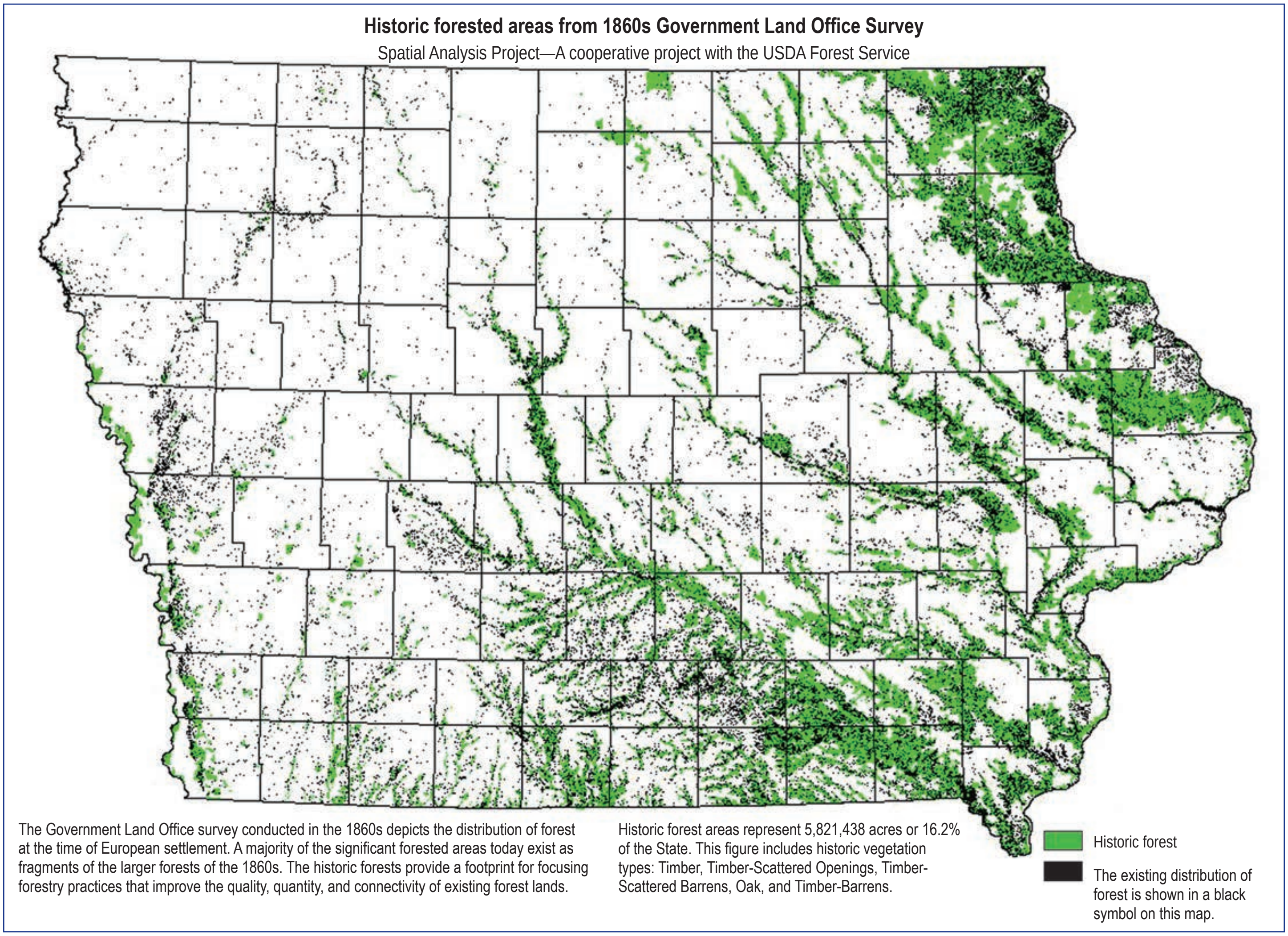

Figure 1. Forested areas in Iowa during the early 19th century. (Source: Kathryne Clark, Geographic Information System [GIS] Analyst, Iowa Department of Natural Resources, using General Land Office maps as surveyed from 1836 through 1859)

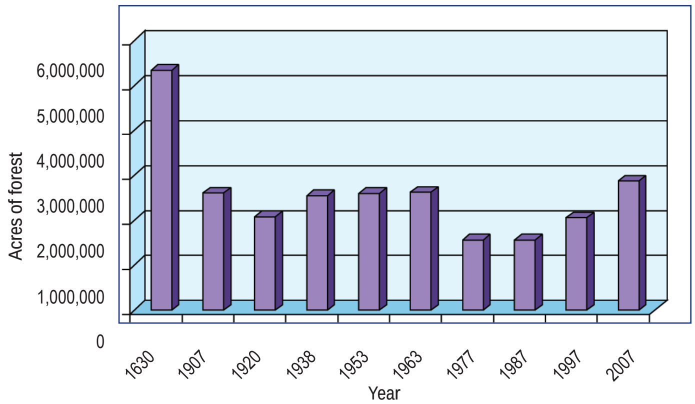

Figure 2. Acres of forest in Iowa, 1630 through 2007. (Source: Smith and others, 2009)

green ash (*F. pennsylvanica* Marshall), sugar maple (*Acer saccharum* Marshall), black maple (*A. nigrum* Michx. f.), and silver maple (*A. saccharinum* L.).

Iowa opened for settlement in 1833, and by 1910, most of the land had been converted to agricultural production. Early settlers used trees for lumber or other wood products or cleared areas to grow agricultural crops. As local populations increased, growing demand for housing materials led to greater use of Iowa's forest resource in the latter half of the 1800s.

While clearing trees to grow food crops was beneficial in many areas, in some locations the clearing of forests led to poor crop yields and severe soil erosion. Small mills were numerous throughout the State to process locally harvested trees, enabling farmers to supplement their income. During this time, steamboats were a popular mode of transportation on the Mississippi, depending heavily on riverbank timber for fuel. This increased timber demand further depleted Iowa's timber resource as well as the quality of Iowa timber during this time.

The lumber industry in Iowa began with the establishment of the first sawmill in 1831 on the Yellow River in the northeastern part of the State. Soldiers from Fort Crawford, under the direction of Lieutenant Jefferson Davis (later to become President of the Confederate States of America), constructed a dam. The lumber was cut using power created from a water wheel. For many years, waterpower was the energy source for the numerous sawmills that multiplied rapidly along the Mississippi River and its main tributaries.

Between 1850 and 1900, the area around the town of Clinton in eastern Iowa was regarded as the sawmill capital of the Nation. Huge log rafts were floated down the river from Wisconsin and Minnesota, cut into lumber at Clinton, then shipped to growing communities east, west, north, and south via the river and the railroads. For a while, Clinton held the reputation as the largest producer of finished lumber in the world and boasted of 17 millionaires in lumbering and related businesses. The lumber was used for producing finished goods such as doors, windows, staircase posts, pillars, moldings, and all sorts of fancy "gingerbread" ornamentation that covered many older Victorian homes in the mid-1800s (Iowa Public Television 1979). In 1865, Iowa sawmills produced 21.5 million board feet (MMBF) of lumber. By 1892, production had risen to more than 195 MMBF.

Trees normally would grow back rapidly after they were cut. But with the invention of barbed wire in 1873, the forests faced another threat as people found it easier to use their woodlands for grazing. Although the livestock did not always destroy the timber, the heavy livestock compacted the soil, ate or trampled seedlings, and changed the character of the woodland flora and fauna. Coal mining also took its toll on forests as trees were cut to shore up mine shafts. By 1900, more than 4.0 million ac (1.6 million ha) of Iowa's original forests had been removed for other uses. A decline in prosperity of the Iowa lumber industry began as desirable timber that had previously been locally harvested and rafted to the Iowa mills became exhausted.

In 1974, the U.S. Department of Agriculture (USDA), Forest Service's Forest Inventory and Analysis (FIA) inventory found that Iowa had reduced the forest land cover to its lowest level ever recorded, at 1.5 million acres. At that time, every county in the State had some forest land, from 25 percent in Allamakee County to less than 1 percent in 31 of the State's 99 counties.

Net Change in Forest Land

Since 1850, 1,051,934 acres of forests have emerged in what are considered to be new locations; another 1,794,958 acres of forest that existed before 1850 are still around today. Iowa had approximately 6,471,581 acres of forest area in 1850, which means that 4,676,623 acres of original forest have been removed since this time. Overall, Iowa has experienced a net loss of 3,624,689 acres of forest, or more than one-half of the forest area in existence at the time of European settlement (figure 3). No data exists to determine if the best quality forest was lost or to describe the composition of the original forests that were not lost. Much of the forest that was removed came from land with relatively high-quality soil for the purpose of crop production.

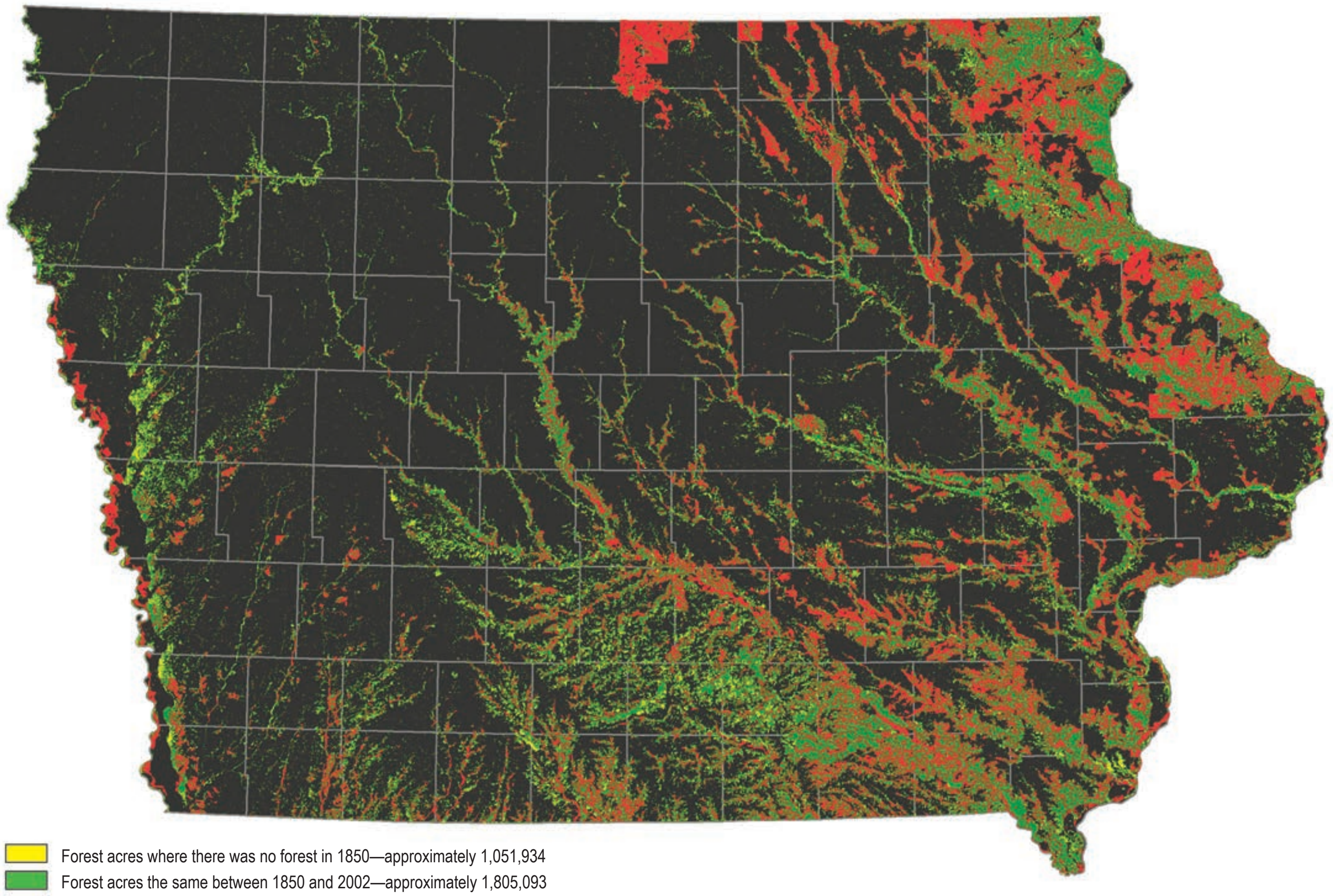

Figure 3. Changes in Iowa's forest land, 1850 through 2002. (Source: Kathryne Clark, Geographic Information System [GIS] Analyst, Iowa Department of Natural Resources, using General Land Office maps as surveyed from 1836 through 1859, Iowa Cooperative soil survey, and Iowa Department of Natural Resources geological survey)

Iowa's Climate

Iowa, located in the heartland of the United States, is bordered by the Mississippi River on the east and the Missouri and Big Sioux Rivers on the west. Iowa has a relatively low relief, with elevations running from a high of 1,670 ft (510 m) above sea level in Osceola County in northwestern Iowa to 480 ft (145 m) above sea level in Lee County in the southeastern corner of the State (NOAA 2013).

Iowa's climate is influenced by its mid-continental location and the sheltering effect of the Rocky Mountains. A wide range of temperatures occur throughout the year, with hot summers and cold winters. Strong winds blow across Iowa throughout the year, which makes any exposed soil vulnerable to wind-blown erosion. This effect is most obvious by the creation of the Loess Hills, located along the State's western boundary.

Iowa often experiences seasonal extremes and frequent local, rapid weather changes because of the convergence of cold, dry Arctic air; moist maritime air from the Gulf of Mexico; and dry Pacific air masses (NOAA 2013). The average temperature in the summer ranges from 71.0 °F (21.7 °C) in the northern part of the State to 73.0 °F (22.8 °C) in the southern part. December to February winter temperatures average 22.0 °F (-5.6 °C), with an average winter difference of 6.5 °F (3.6 °C) between north and south. Temperature minimums of -25.0 °F (-31.7 °C) are not uncommon in northern Iowa (NOAA 2013).

These climatic factors combine to influence the length of the growing season across the State. Late spring frosts and early fall freezes reduce the growing season to 135 days in northern Iowa. The longest growing season is in southeastern Iowa, with an average of 175 days.

The northwest part of the State is the driest, with an annual precipitation of 28 in (71 cm), while the southeast is the wettest, with an annual precipitation of 36 in (91 cm) (NOAA 2013). Statewide, winter snowfall averages 32 in (64 cm).

Northern Iowa receives frequent, often blowing and drifting snow typically associated with strong winds. Southern Iowa may experience substantial snowfall but has more frequent ice storms resulting in a snow cover that is often covered by a surface crust of ice or hard snow. Harsh conditions seldom remain for more than a few weeks in most of the State, particularly in the southern half.

Like most States, periods of severe drought and periods of excessive precipitation can have a dramatic impact on terrestrial and aquatic vegetation and on their associated fish and wildlife species. Every 30 years or so, a drought period occurs that remains for several years. The most famous drought was in the 1930s, when the Plains States were called the "Dust Bowl." Two "100-year" floods (1993 and 2008) caused billions of dollars in damage to private property and wiped out habitat for a variety of wildlife species. Tree mortality increased for riparian species like silver maple, cottonwood, and black walnut in the 1990s and it is expected that the trend will regain momentum in the upcoming decade as a result of the 2008 flooding.

Iowa's Land Distribution and Ownership

Forest cover in Iowa is now about 3.1 million ac (1.3 million ha), or 8 percent (figure 4). Land used for agricultural crops represent 58 percent of the land usage, with an additional 4 percent being idled in the Conservation Reserve Program (CRP). Prime agricultural farm land is primarily located in the northern half of the State, much of it along river valleys. The conversion of Iowa's native ecosystems in the past enables the State to produce one-tenth of the Nation's food supply.

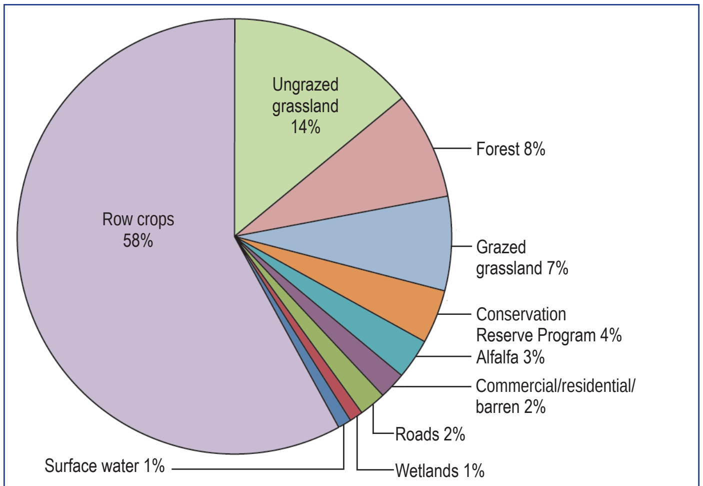

Figure 4. Iowa's land composition by percentage, 2002. (Source: Landsat Thematic Mapper Satelite Imagery)

Public Forest Land

The Iowa Department of Natural Resources (DNR) purchases land to manage and protect natural resources, to maintain unique ecosystems for future generations, to maintain a pool of biodiversity for future generations, and to provide recreational opportunities to all the people of the State. Through their land acquisition program, wetlands, forests, scenic areas, prairies, wildlife and fish habitat, rare species habitat, and other resources are being protected and managed. Public areas are important for maintaining the State's native biological diversity, which is often much harder to preserve on private lands.

In 2008, Iowa had 816,000 ac (330,220 ha) of area in public ownership, of which slightly less than 637,000 ac (257,800 ha) were classified as land (figure 5). Within the land category, 44 percent is classified as forest. In 2002, public agencies owned more than 9 percent of the forest land in Iowa, only a slight increase from 8 percent in 1974. Public forest land allows for different management activities, depending on which bureau within the DNR (Forestry, Parks, or Wildlife) oversees a certain property.

The State Forestry Bureau manages 34,597 ac (14,000 ha) of forest on its 45,230 ac (18,300 ha) of public land, with the remaining areas in roads, lakes, prairie, or cropland. State forest areas are subdivided into 10 State forests that represent all of the major forest habitat types of Iowa along with a range of ages. These forests are mainly managed for timber production, wildlife habitat, water quality, and air quality.

The State Parks Bureau has 31,703 acres of forest on its 57,754 acres of public land. Some of these areas have some of the oldest trees in the State growing on them. The State Wildlife Bureau has the largest holding of forest, with 94,547 acres within its 347,852 acres of public land.

Forest management is permitted on areas owned by the State Wildlife and State Forestry Bureaus. Parks and preserves generally do not practice active management, an approach that allows for natural selection on their properties. Salvage sales are an exception; they often take place after strong windstorms, flooding, or tornados cause damage to their resources.

Other large public landowning bodies are the 99 County Conservation Boards, which collectively own more than 143,000 ac (57,870 ha) of property, of which 65,354 ac (26,448 ha) are forested.

Federal agencies own 190,000 ac (76,890 ha) of land within Iowa, of which 37,632 ac (15,230 ha) are forested. The U.S. Army Corps of Engineers and the U.S. Department of the

PUBLIC LANDS	
Manager	Acres
Department of Natural Resources:	
Wildlife	347,852
Parks	57,754
Forestry	45,230
Fisheries	18,050
Law Enforcement	158
Unknown	1,682
County Conservation Board	143,176
Army Corps of Engineers	112,502
U.S. Fish and Wildlife Service	78,532
Cities	5,045
The Nature Conservancy	6,680
White Rock Conservancy	4,171
National Park Service	2,709
Iowa Natural Heritage Foundation	1,814
University of Iowa	1,068
Iowa State University	307
Other	819
Wetland Reserve Program	100,000
Waterfowl Production Areas	20,216

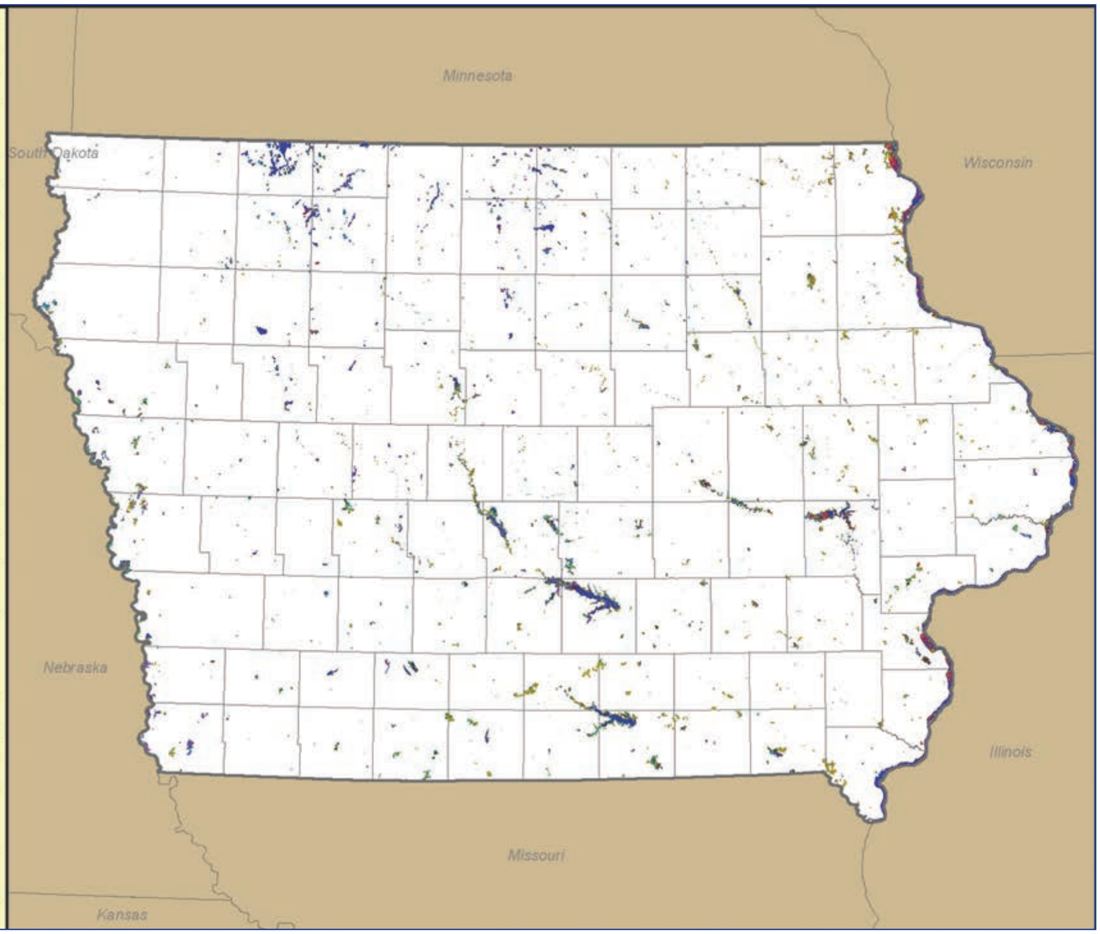

Figure 5. Iowa public land composition in acres, 2002. (Source: Kathryne Clark, Geographic Information System [GIS] Analyst, Iowa Department of Natural Resources, using satellite land cover from 2002 and public lands database)

Interior, U.S. Fish and Wildlife Service, own the most Federal land. Iowa has a smaller proportion of public land than almost any other State in the country and has no national forests. The Federal agencies that do own property manage their land for wildlife refuges, flood control, and navigational systems with accompanying recreation areas.

About 40 percent of the acres of publicly owned land are on highly erodible soils, indicating a need for permanent vegetation on these areas to improve water quality, stabilize soil, and improve habitat for wildlife. The average corn suitability rating for the land owned by the DNR is 32 on a scale of 100, indicating that most DNR-owned land is not suitable for agriculture.

Private Land

Privately owned woodlands have decreased dramatically in size since the middle of the 20th century. In 1954, the average woodland owner owned 45 ac (18 ha) of woodland; this number declined to 31.0 ac (12.5 ha) in 1990 and 17.0 ac (6.9 ha) in 2003. In 2006, most forest landholdings were less than 9.0 ac (3.6 ha); moreover, the number of private woodland landholdings nearly tripled from 55,000 in 1990 to 150,000 in 2008 (Butler 2008). These numbers are alarming because they reflect the extent to which interior forests have been reduced over time and the extent to which they may be reduced in the future.

Forest Cover Types

Iowa forests are 98 percent hardwoods (figure 6). The shade-intolerant white oak-red oak-hickory forest type represents the largest forest type. The second most prevalent forest type is mixed upland hardwood. Shade-intolerant black walnut represents a small but economically important position in Iowa’s forests (figure 7). The only native conifers in Iowa are white pine (*Pinus strobus* L.), eastern red cedar (*Juniperus virginiana* L.), and balsam fir (*Abies balsamea* [L.] Mill.). Tree species diversity is highest in eastern Iowa and decreases as moving west. Because of the prevalence of wildfire before statehood, most trees in the State today are fire-adapted species; however, with the suppression of fire that accompanied Iowa's settlement, thinner barked, shade-tolerant trees have been able to grow within the dominant oak-hickory forest type.

Iowa's State tree is the oak, although it is not specific to 1 of the 11 oak species native to the State. White oak (*Quercus alba* L.) and bur oak (*Q. macrocarpa* Michx.) trees are typically the oldest living oak species, with some exceeding 400 years in age. Oaks are disturbance-dependent species, meaning that they have a competitive advantage over other trees in areas susceptible to wildfire. The oak-hickory forest type is the largest in Iowa; however, this forest type has declined in recent years, from 37 percent of total forest area in 1990 to 26 percent in 2008. Lack of active management and disturbance on private and public forest lands are the leading causes of oak-hickory forest decline in Iowa.

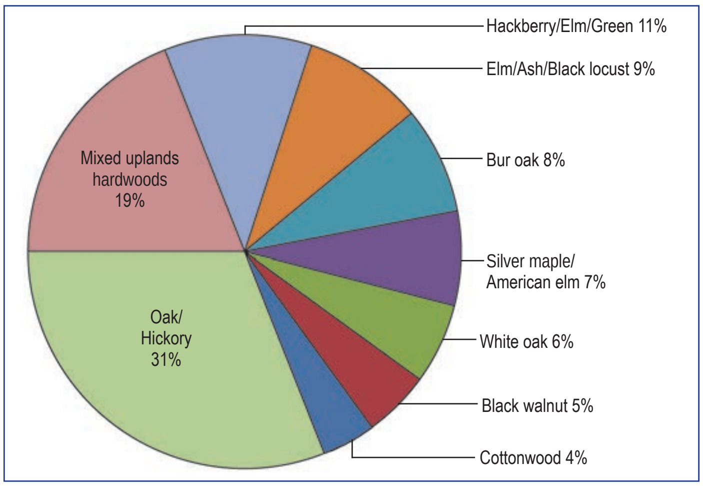

Figure 6. Forest type as a percentage of total forest in Iowa, 2008. (Source: Miles, 2010)

Figure 7. Black walnut is Iowa's most valuable timber species. (Photo by Paul Tauke, Iowa Department of Natural Resources)

Forest Management in Iowa

Iowans are aware their forest resource has economic potential by harvesting timber when income is needed. This harvesting method does not lead to sustainable reproduction of traditional forest types that have existed in Iowa. On the other end of the spectrum, leaving the forest alone by "doing nothing" has consequences, since natural management regimes, like fire, have been removed from the landscape. The attitude that the forest can regenerate itself is a continual challenge to overcome when convincing people that the condition of Iowa's forest resource is in decline.

Within the Forestry Bureau, 16 district foresters are dispersed throughout the State to help the more than 150,000 private forest landowners manage their forest land and successfully establish tree plantings. In total, four area foresters and nine natural resource technicians manage Iowa's four State forests. District foresters and area foresters are supervised by the private lands forest supervisor and the State forest section chief, respectively. Four more specialized foresters oversee forest health, fire, urban, and special projects issues. The State Forest Nursery is also managed by the private lands forest supervisor as well as a secretary, a nursery forester, three natural resource technicians, and an inmate crew capable of growing and shipping up to 4 million tree seedlings per year. Finally, the Forestry Bureau as a whole is under the direction of the Forestry Bureau chief.

State Funding for Forestry

The Forestry Bureau has five general sources of funding: (1) general fund income, which is allocated by the State of Iowa through the Legislature and Governor's Office; (2) Federal funding, provided by the USDA Forest Service to support priority programs; (3) conservation funding, generated by the State Forest Nursery; (4) the Forest Enhancement Fund, which provides $0.05 for every seedling sold to support district forester positions in northeast Iowa; and (5) partner funding from organizations such as Alliant Energy, MidAmerican Energy, Black Hills Energy, Trees Forever, Iowa Woodland Owners Association, Iowa Tree Farm, and Iowa Bankers Association. In 2010, funding from the five sources provided 40, 22, 25, 5, and 8 percent of the Forestry Bureau's budget, respectively (Iowa Department of Administrative Services—State Accounting Enterprise 2010).

The budget for the bureau was about $5.5 million per year for fiscal years (FYs) 2008 and 2009. Because of across-the-board budget cuts, the Forestry Bureau lost more than $550,000 in general funding during FY 2010, though it received an increase in Federal funding of approximately $250,000. Overall, the Forestry Bureau saw a net decline of roughly $367,000 from former levels during FY 2010.

General fund dollars are especially important for use in matching Federal funding, and there could come a point when not enough general fund dollars are available to match available Federal funds. The DNR State Forestry Bureau currently is able to bring in $1.86 to $2.05 of Federal funding for every general fund dollar it receives. Partner funding is dedicated to producing educational materials for the Trees for Kids and Trees for Teens programs, and most of such funding goes toward residential tree distribution programs.

Iowa's State Forest Nursery

The State Forest Nursery in Iowa was originally established in Ames in the 1930s by the Civilian Conservation Corps. The land was later purchased by the Iowa Conservation Commission in 1940. The nursery is operated by the Forestry Bureau and provides low-cost native tree and shrub material to encourage more planting in the State. Without it, forest landowners would be forced to pay more to plant trees on their property and, in many cases, would likely revert to buying their trees from out-of-State nurseries to get lower prices. The nursery has sold more than 150 million bareroot seedlings since its establishment, including 64.5 million in the past 20 years (figure 8, table 1).

In addition to bolstering the State's economy, use of native tree material ensures that insects and diseases that are not established are not brought in; moreover, seedlings from outside Iowa may not be as adapted to the State's climate and may therefore be more susceptible to such problems because of stress. Nonnative seedlings are often less productive at growing wood and mast (forage for wildlife) as well. Flowering schedules may be off for species brought into Iowa, causing those trees to be unable to produce fruit or seed, which affects food for wildlife and limits reproduction for that species.

The State Forest Nursery has a main central facility in Ames and another growing facility in Montrose. The Montrose operation is located on 45 ac (18 ha) and has 23.0 ac (9.3 ha) in production. The sandy loam soils at this site are ideal for growing conifers and hardwoods. The site is approximately 3 hours south of the Ames facility, allowing for earlier lifting in the spring and later lifting in the fall, which helps get more grading done when the soils at the Ames facility are frozen. Only one full-time person staffs this facility, which can grow

Table 1. Average seedling sales for top 10 seedling species grown at the Iowa State Forest Nursery from 2005 through 2010.

Black walnut	150,000
Red oak	135,000
White oak	115,00
Swamp white oak	95,000
Bur oak	90,000
Eastern Red cedar	85,000
White pine	70,000
Silver maple	60,000
Ninebark	55,000
Wild plum	50,000

Source: Aron Flickinger.

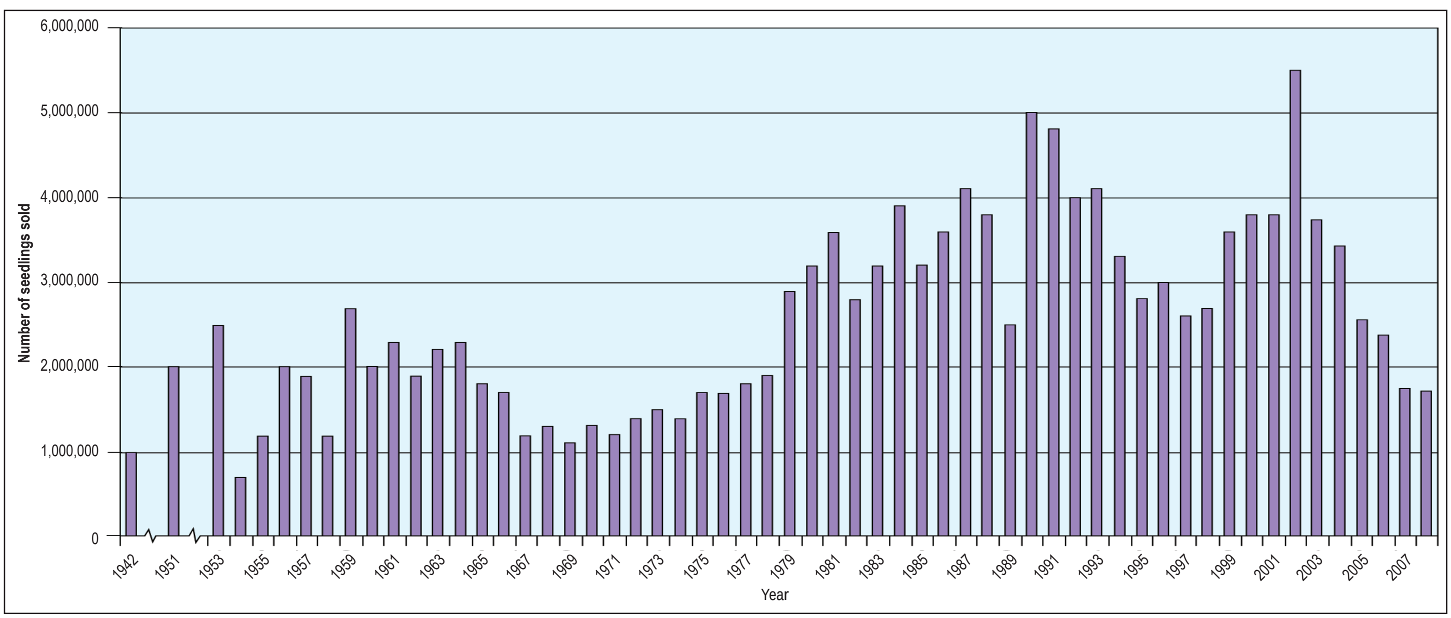

Figure 8. State Forest Nursery seedling sales, 1942 through 2007. (Source: Aron Flickinger)

up to 1 million seedlings annually. The labor force comes from a nearby correctional facility to help during the lifting and weeding seasons. The facility has an onsite orchard with hazelnut (*Corylus americana* Walter), white cedar (*Chamaecyparis thyoides* [L.] Britton, Sterns & Poggenb.), serviceberry (*Amelanchier arborea* (Michx. f.) Fernald), and green ash seed to help reduce the cost of purchasing seed.

The Ames location has 100 ac (40.5 ha) of land with 45 ac (18.2 ha) under production. At Ames, 2 to 4 million trees are grown annually, including most of the shrubs and many of Iowa's native hardwood species. The soils at Ames are heavier than at Montrose, which can create challenges for growing good root systems and causes more wear on equipment. The main building was built in 1975 and, through the years, a total of 10,000 square feet of refrigeration has been added onto the grading room, which has increased the storage capacity to 1.5 to 2 million seedlings. This cooler allows for more fall lifting to allow more seedlings to be available for filling customer orders when the weather is suitable in the spring.

Iowa Code specifies that the nursery's budget for growing costs be dependent on its seedling sales within a particular fiscal year, which makes for serious financial stress during years with poor sales. State rules and economic restraints can also make it difficult for the nursery to market its products and cover its operating costs.

Nursery Growing Season

Growing good-quality seedlings begins with soil tests in the nursery lab. Samples are taken during the summer to determine soil needs. Fertilizers, pH adjusters, and organic matter are then added to the soil as necessary. In early fall, some seedbeds are fumigated to remove disease problems and reduce weed populations.

Most of the seed for the 50 species of plants grown are purchased from seed collectors, but a growing percentage of seed is collected by nursery personnel. Seed orchards are continually being established to protect native seed sources and to provide more accessible seed for future planting needs. Most seeding is done in the fall. The seeds are pressed into freshly tilled soil and covered with a mulch of ground corn cobs. The seeds germinate the next spring as soon as the soil warms up sufficiently.

Supplemental water is added through the irrigation system when necessary. Fertilizers are added at required intervals throughout the growing season. Weeds are controlled mainly by chemical means, although some mechanical and hand weeding is also necessary. Insect and disease problems are diagnosed and handled on an individual basis as needed. Seedlings are root pruned after they reach saleable height and the soil is 50 to 70 °F (10 to 20 °C). All of these steps together maintain good seedling growth and vigor; along with a balanced root-to-shoot ratio.

Most of the hardwoods and shrubs grow 1 year before being sold; the conifers take 2 or 3 years (depending on the species) to reach saleable height. As many seedlings are harvested in the fall as weather permits; the remainder are harvested in the spring. Orders for seedlings are shipped out in the fall or spring, based on customer request. The seedlings are harvested by a machine that cuts the roots at a depth of 6 to 10 in (15 to 25 cm) and, with several people assisting, shakes the dirt from the roots. The seedlings are then placed in plastic-lined crates and taken into a cooler, where they are stored until they can be counted, sorted, and sealed in polyethylene bags. The seedlings are then stored in another cooler until they are prepared for individual orders and shipped throughout the State within wax-lined paper bags.

Tree Planting in Iowa

The reasons for planting trees are numerous. Between 1,000 and 2,000 people buy trees from the State Forest Nursery each year. Most people are planting trees as part of a conservation practice offered by the USDA's Natural Resources Conservation Service (NRCS) or Farm Service Agency (FSA). People who qualify for these programs own land that is adjacent to important water corridors or have highly erodible land that they want to protect. An increasing number of people own smaller pieces of land for either an acreage to live on or a place to view or hunt wildlife.

In Iowa between 1998 and 1999, about 3,630 ac (1,470 ha) of trees were planted, which ranked the State number 6 for tree planting out of the 20 Northeastern States (figure 9). During years in which conservation programs promoting tree planting are particularly successful or widespread, State Forest Nursery sales are typically above average. Conversely, when conservation programs cannot compete with commodity prices, tree sales go down. With a legislatively mandated requirement to operate at the cost of growing trees, the viability of the State Forest Nursery is a challenge because demand for seedlings is dependent on many programs outside of its control.

Private landowners have responded positively to market

Figure 9. Native conservation seedlings from the State Forest Nursery planted on private land. (Photo by Bruce Blair, Iowa Department of Natural Resources)

incentives and government programs, including subsidized afforestation on unproductive agricultural land, which is one reason that forest land has increased in the State in recent years. The ability of the State Forest Nursery to supply large quantities of native nursery stock at a relatively low cost has provided Iowans with excellent opportunities to develop forests on their land. Without the nursery, Iowans would have had to pay more for their seedlings, which would have left them with fewer resources for weed control and other activities critical for successful tree planting establishment; if the price had increased, it is likely that fewer acres would have been planted during this time period. It is important that promotions of tree planting continue to ensure that landowners stay in touch with their properties and leave legacies for future generations. This connection with the land is especially important when considering the extent to which landholdings have shrunk in the past half-century.

Conservation Programs and Incentives for Forest Landowners

Roughly 90 percent of Iowa's forests are privately owned, and Iowa DNR foresters work with approximately 2,000 forest landowners annually. Interactions between foresters and landowners begin with evaluations of forest resources, discussions of forest landowner objectives, and consideration of forest management alternatives. District foresters provide free consulting services for forest landowners. They can work with landowners to apply for tree planting cost-share assistance at local FSA or NRCS offices to reduce the cost to the landowner for tree planting, forest stand improvement, and wildlife habitat improvements. Landowners can receive stewardship plans to provide frameworks for achieving their management goals and objectives in sustainable ways. Meeting with private landowners gives professional foresters the opportunity to provide education about the benefits of proper long-term forest management, and in a State for which agriculture is the most lucrative way to make money from land in the short-term, these interactions are especially important.

A variety of conservation programs support forestry practices in Iowa (figure 10). Conservation programs that encourage tree planting can be a disadvantage when competing with agriculture, which generates income more quickly and consistently; however, lowering the input costs of tree planting is one way to make forest-related activities more economically feasible.

The Forest Land Enhancement Program has not received funding for private forest landowners to improve their woodlands that it was originally to receive, and, as a result, the program no longer exists. Only $146,000 was available to Iowa in 2003, and funding has decreased more in subsequent years.

The Wildlife Habitat Incentive Program (WHIP) began in 2003 and, for the most part, has provided steadily increasing funding for Iowa (from $52,000 in 2003 to $93,000 in 2006). This Federal program is administered through the NRCS, with technical assistance provided by foresters, wildlife biologists, or NRCS staff. Programs eligible for this funding assistance include tree planting, forest stand improvement, and brush management.

The Environmental Quality Incentives Program (EQIP) has provided variable funding for forestry practices through the years and has provided funding for projects similar to those funded by WHIP. In 2001, more than $288,000 was provided to forest landowners, the most offered in any year through 2006. In 2009 and 2010, approximately $500,000 per year of EQIP funding was set aside for forestry practices on private lands.

Resource Enhancement and Protection (REAP) is a State program that provides funding for forest landowners to get trees planted or to improve the woodlands on their property. As its name implies, REAP invests in the enhancement and protection of the State's natural and cultural resources. Iowa has an array of natural and cultural resources, and REAP is likewise diverse and far reaching. Depending on the individual programs, REAP provides money for projects through State agency budgets or in the form of grants. Several aspects of REAP also encourage private contributions that help accomplish program objectives. REAP is funded from the State's Environment First Fund (Iowa gaming receipts) and

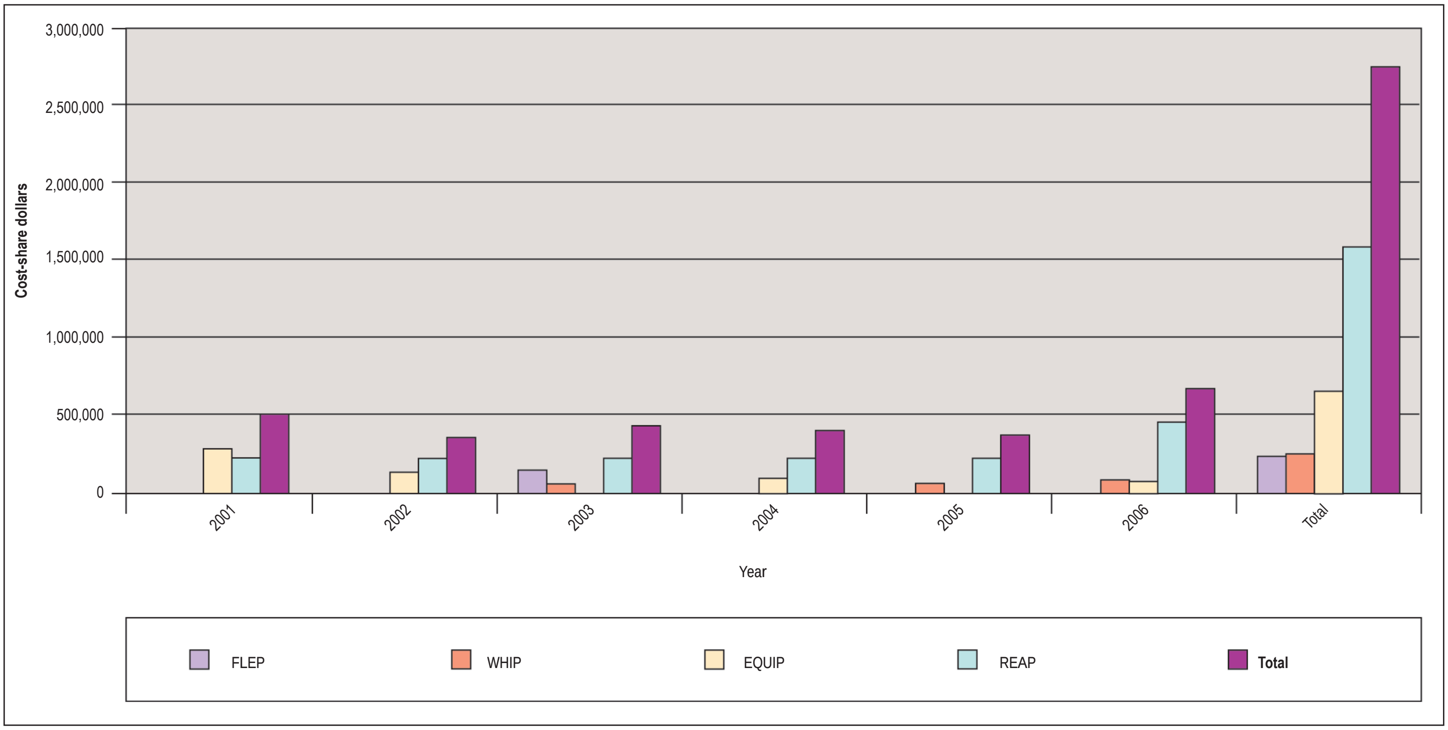

Figure 10. Cost-share dollars spent on forestry practices per program, 2001 through 2006. EQIP = Environmental Quality Incentives Program. FLEP = Forest Land Enhancement Program. REAP = Resource Enhancement and Protection. WHIP = Wildlife Habitat Incentive Program. (Source: Paul Tauke, Iowa Department of Natural Resources)

from the sale of the natural resource license plates. From 2001 to 2005, an allocation of $225,500 was available annually for forestry practices; that amount increased in 2006 to $473,000.

A summary of CRP enrollment from the July 2009 report shows that Iowa had the most rental payments of any State with $197,520,000. These rental payments were associated with 105,241 contracts on 52,965 farms protecting 1,705,312 acres. Within the protected areas, 28,550 acres, or 1.7 percent of CRP acres, were planted for trees. If 700 trees were planted on each of these 28,550 CRP acres, nearly 20 million total trees would be planted. A program like CRP benefits water quality and provides long-term soil protection on highly erodible soils. Landowners are less likely to remove trees after a 15-year contract, and the tree planting provides society a better return than that provided by agriculture, because those trees will continue to protect the soil and water, sequester carbon, and provide wildlife habitat. Trees make sense for long-term protection of sensitive land because, after being established, they are more difficult to remove; planting grass provides many good benefits but may not provide them for the same amount of time because it is much easier to remove. Nurseries that provide conservation seedlings and consultants who plant these seedlings for landowners benefit from tree planting incentive programs as well.

The amount of land enrolled for conservation practices by the NRCS increased between 2002 and 2004 in Iowa (table 2); however, a decrease in the number of acres of trees actually planted also occurred during the same time. Tree planting represented only slightly more than 1 percent of the conservation acres funded by the NRCS; the permanent establishment of woody vegetation is something most farmers steer away from, as grassland is much easier to establish, maintain, and, if so desired, reconvert to agricultural land. A lot of potential acreage for conservation exists in Iowa, however, the preferred type of conservation is some type of grass cover.

Oak-Hickory Regeneration

Since 1954, Iowa has been losing more than 7,000 acres of oak-hickory forest annually (Miles 2010). As a result, oak-hickory management is a priority in several areas of the State

Table 2. Base conservation by USDA Natural Resources Conservation Service in Iowa, 2002 through 2004.

	2002	2003	2004
Acres planned for conservation	1,174,262	1,153,154	1,440,157
Acres of trees planted	6,399	4,398	3,518
Highly erodible land treated (acres)	405,678	381,708	

Source: http://www.ia.nrcs.usda.gov/programs/conservationoperations.html (March 2010) (figure 11). Some areas are relatively easy to manage because of their high concentrations of oak trees; areas that used to be oak-hickory but have made the transition to other, mostly shade-tolerant, species are much harder to restore to oak-hickory. While it is important to ensure that the latter areas remain as forests, the sheer amount of resources that would be necessary to restore them to oak-hickory forests makes such a task impossible.

Managing native vegetation communities in Iowa is a challenge because of the State's highly fragmented forests and near-complete removal of historical disturbance regimes like fire. Active forest management is now needed to help oaks adequately regenerate in Iowa's maturing forests; whenever possible, the DNR State Forestry Bureau actively manages oak in State forests using even-age silvicultural techniques.

An ecosystem's forest type affects the wildlife habitat, herbaceous cover, wood products, recreational opportunities, and economic value of that ecosystem. Wildlife that depend on oak-hickory trees for habitat and food may not be able to survive without them; many of the common herbaceous plants found in oak-hickory stands cannot tolerate heavy shade; outdoor recreation enthusiasts looking for enjoyment from the wildlife and plants usually found in an oak-hickory forest may not receive the same level of satisfaction from shade-tolerant forests; and, finally, without oak trees, the livelihood of sawmills will be threatened.

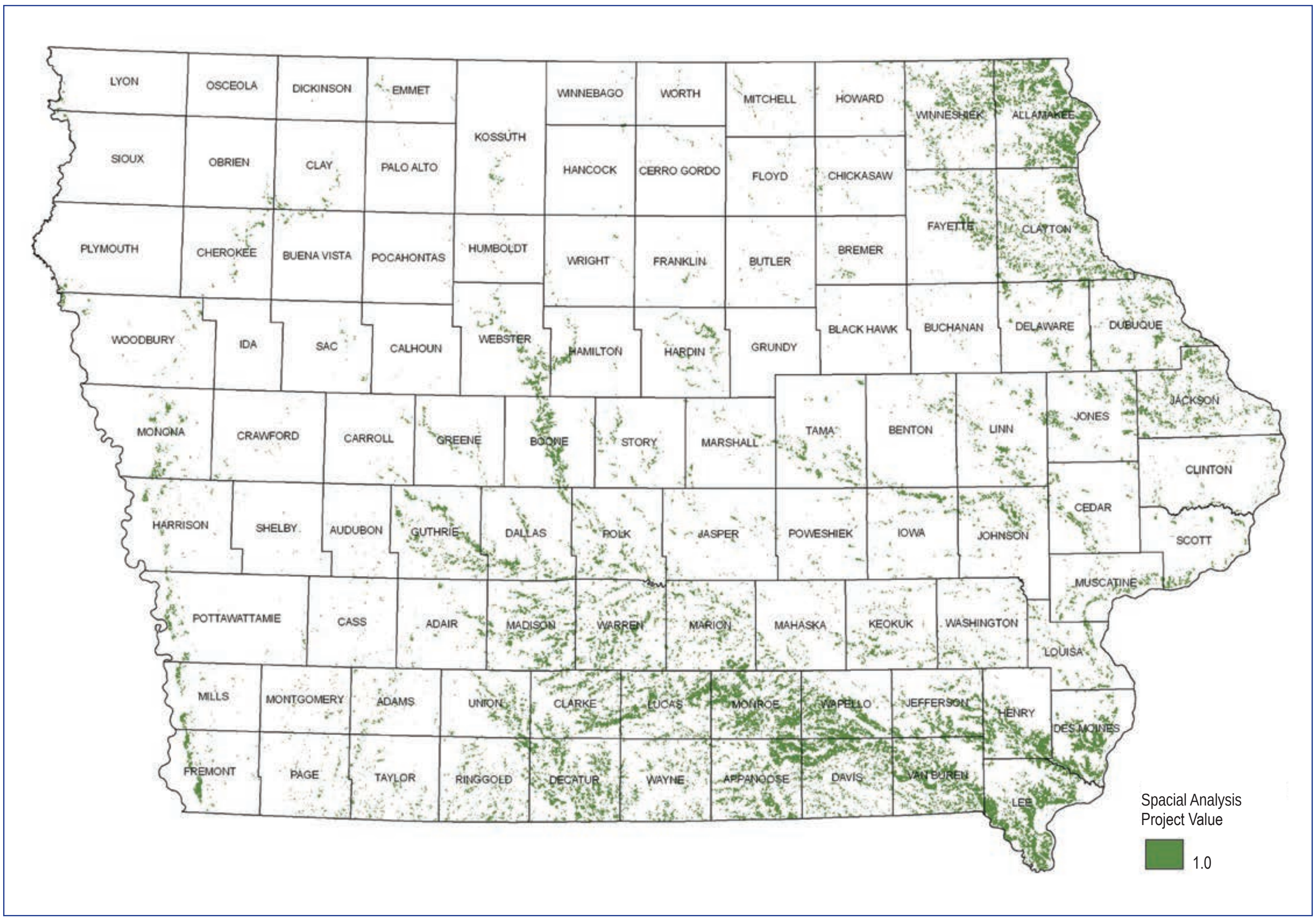

Figure 11. Priority areas for oak regeneration. (Source: Kathryne Clark, Geographic Information System [GIS] Analyst, Iowa Department of Natural Resources, using 2008 Forest Inventory and Analysis data and satellite land cover from 2002)

Preserving Tree Genetics

The goal of Iowa's tree improvement program is to preserve the genes of locally adapted trees. Maintaining a pool of genetic diversity for all native species on sites located across the State would ensure that Iowa's trees are in a suitable position to withstand climate change and threats from disease and insects in the future; this pool would also provide a dedicated seed source to supply future seed needs for nurseries to ensure a native local seed source is available.

The tree improvement program has collected from a diverse gene pool of black walnut trees in Iowa. As the most valuable black walnut trees are harvested, branches are collected to propagate seedlings with identical genetics. This collection will give landowners a better pool of trees from which to choose for growing and will have positive implications for future yield and genetic and biological diversity; after enough of these trees are selected, the sample of genes will also be large enough to represent more than 95 percent of the genetic variation within this species. Since 2003, the program has been testing for a fast-growing black walnut tree capable of growing above vegetation and wildlife browsing lines to quickly capture a site. The most successful tree so far, which is being reproduced and tested in field trials, experienced growth of almost 9.0 ft (2.7 m) in 2 years and 25.0 ft (7.6 m) in 5 years.

The other focus of the tree improvement program is to preserve the genes of the native butternut (*Juglans cinerea* L.) in an effort to prevent its extinction from butternut canker (figure 12). Branches are collected from native trees and then grafted onto walnut root stock in an effort to maintain a population of native Iowa butternuts. Seedlings from 20 Iowa trees and more than 100 trees from other States are being tested at the Loess Hills State Forest in western Iowa and Yellow River State Forest in northeast Iowa (the latter site is in an area of the State that is still highly susceptible to the disease, while the former site is outside of the butternut canker range).

Figure 12. Butternut seedling established at Loess Hills State Forest. (Photo by Aron Flickinger)

Challenges to Tree Planting in Iowa

Forest Health

Iowa forests contain more than 1 billion trees from 68 species. Of these trees, 25 percent are susceptible to fatal insect or disease problems, such as oak wilt, oak decline, emerald ash borer, Dutch elm disease, and pine wilt. Iowa's forests are facing an unprecedented level of native and invasive pests that threaten to create a new wave of mortality unseen since the arrival of Dutch elm disease. While most forests are relatively diverse, these threats will have a substantial impact on the composition of the State's forests and urban tree canopies in the future. Each year, the Iowa DNR State Forestry Bureau cooperates with numerous agencies to protect Iowa's woodlands from insects, diseases, and other damaging agents. In a recent report the DNR identified five key pests that have emerged as a severe threat to Iowa's native woodland and community: gypsy moth, emerald ash borer, bur oak blight, thousand cankers disease of black walnut, and Asian longhorned beetle (Iowa DNR 2012).

Gypsy moth catches were at an all-time high in 2010, exceeding the previous record by a factor of more than three times. As a result, more than 170,000 acres of forest land were treated in 2011 to reduce the exploding population. The populations were reduced and only 225 Gypsy moths were captured in 2012. In 2010, emerald ash borer was found in Iowa, resulting in a quarantine that placed restrictions on how far ash wood material, including firewood, could be moved. In 2012, emerald ash borer was found in four new sites within Allamakee County. This county remains the only quarantined county. Bur oak blight (figure 13), identified in Iowa in 2007, has continued to spread and cause advanced decline and premature mortality for bur oaks in rural woodlands and community forests. Thousand cankers disease of black walnut has not yet been identified in Iowa. The Iowa DNR is actively monitoring for the walnut twig beetle, however, which carries thousand cankers disease. Asian longhorned beetle has not been identified in Iowa, the locations that have this pest have been devastated. Quarantines are in place to help prevent the spread of and eradicate the beetle. These five emerging pests will place an additional financial burden on Iowa's communities by threatening nearly all 26 million community trees.

Figure 13. These bur oak trees show different levels of decline; the tree on the right is healthy, the tree on the left is infected with bur oak blight (Tubakia), and the tree in the top left corner is dead. (Photo by Aron Flickinger)

They threaten 55 MMBF (56 percent) of the wood products volume that is currently desired for harvesting and more than 6 billion board feet (53 percent) of the existing timber volume standing in Iowa's forests today.

Leaf tatters causes a reduction in interveinal leaf tissue in newly emerged oak leaves as they grow larger, which makes them look deformed or "tattered"; the first sign is curling of the young succulent white oak leaves (figure 14). Not all trees develop tatters, as leaves must be exposed to certain conditions after they have emerged from their buds and may escape tatters if they have grown a certain amount; however, oak trees of all ages growing in both urban and rural areas are susceptible to damage. Leaf tatters was first reported in Iowa, Indiana, and Ohio in the 1980s and in Wisconsin and Minnesota more recently.

Figure 14. White oak leaves protected with pollination bags; the rest of the leaves on the trees showed tatters. (Photo by Aron Flickinger)

Animal Depredation

Where the deer population is high, deer browsing can impact plant species composition and community structure (figure 15). Deer browsing has a profound impact on the establishment of regeneration, the density of hardwood seedlings, and the presence of understory plants. Their impact is reducing biodiversity and hurting Iowa's largest forest type, oak-hickory, the very habitat they depend on in the fall and winter for food and shelter. Deer also impact vegetation by moving parasites and invasive plant seeds, through bedding and by rubbing their antlers on trees. In the winter, they seek shelter in forests, while during the growing season they feed on the herbaceous portion of woody plants under the shade of trees.

Species that fall victim to browsing are unable to regenerate, while those that are not browsed on, including invasive plants such as garlic mustard, continue to thrive. Over time, this selective browsing can lead to a reduction in forest biodiversity, which can then lead to a change in habitat. Reductions in understory plants, for example, can lead to declines in insect activities, including those of pollinators. Browsing activity also affects moisture at the forest floor and the vertical structure within the forest. Soil moisture and humidity decline as more light is able to reach the ground and heat up the area.

Rabbits and mice can also do damage to new tree plantings. They seem to show a preference for oak species, particularly white oaks. Rabbits eat the terminal leaders of seedlings during the winter, and their preference for oaks causes them to fall behind the growth of other tree species in plantings. Mice girdle seedlings during the winter, and their preference for oaks also causes these trees to fall behind the growth of other species (figure 16).

Figure 15. Deer damage to a young conifer planting. (Photo by Bruce Blair, Iowa Department of Natural Resources)

Figure 16. Tree shelters are now necessary for establishing seedlings in some parts of the State, but they provide new habitat for mice as well. (Photo by Bruce Blair, Iowa Department of Natural Resources)

Funding

Perhaps the biggest challenge to Iowa's natural resources may be a lack of funding. The Iowa DNR represented only 0.34 percent of the overall State budget in 2008, and the department experienced further cuts in 2010. Within the DNR, the State Forestry Bureau portion of the general fund was $2,045,015 or 0.0328 percent of the State budget for FY 2010 (CAFR 2010). On average, Iowa taxpayers each contribute $7.82 of their tax bill to Iowa's natural resources each year; of this $7.82, $0.84 goes to the DNR State Forestry Bureau. For every $685 collected in income taxes, $0.25 will go toward Iowa's forest resource.

Iowa's Outlook for Tree Planting Into the Future

Iowans enjoy many attributes of their trees or forests. This enjoyment is shown by the increasing number of acres of forest cover during the past two decades across the State, an increasing housing market on acreages (land with trees or grass), and the number of people visiting local conservation areas for recreation as they turn to local options for vacationing. Because of the high value of Iowa's land for crop production, the future for tree planting will fluctuate.

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Tree Planting in Idaho

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Abstract

Idaho has more than 21.4 million ac (8.6 million ha) of some of the most diverse forests in the Rocky Mountains. The largest part (76 percent) of Idaho's forests is managed by the U.S. Department of Agriculture (USDA), Forest Service, but, progressing north, forests owned by families, the State of Idaho, and forest product companies are increasingly more prominent. Most ownerships seek to reduce stand density and shift species composition to reduce fire risk and insect and disease issues. Idaho has a strong tree improvement program, originating from efforts to develop blister rust-resistant western white pine seedlings. Idaho has two USDA Forest Service seedling nurseries, a nursery managed by the University of Idaho (UI), and a few private seedling nurseries. Highly varied sites present likewise varied challenges to Idaho tree planting. Common threats to seedling survival include seedling moisture stress; rodents (particularly pocket gophers); deer, elk, and moose; and white pine blister rust.

Idaho Forests

Idaho's more than 21.4 million ac (8.6 million ha) of forested land (Witt and others 2012) comprise roughly 40 percent of the State's land area (figure 1). Most of Idaho's forests are located in three "ecoprovinces" (Bailey 1995):

- Northern Rocky Mountain Forest—Steppe-Coniferous Forest Alpine Meadow Province in the northern portion of the State.
- Middle Rocky Mountain Forest—Steppe-Coniferous Forest Alpine Meadow Province in the central portion of the State
- Southern Rocky Mountain Forest—Steppe-Coniferous Forest Alpine Meadow Province in the southeast portion of the State.

Some of the most diverse forests in the Rocky Mountains occur in Idaho. Northern Idaho has a mild maritime influence, which brings significantly more moisture to the northern end of the State than is found in southern Idaho. In general, the most productive and actively managed forest lands are found in the Northern Rocky Mountain Forest—Steppe-Coniferous Forest Alpine Meadow Province.

Idaho's exceptionally rugged topography means elevation and aspect have a large influence on volume of precipitation and its availability to trees. In general, more moisture is available throughout the growing season at higher elevations and on north- and east-facing aspects than at lower elevations and south- and west-facing aspects.

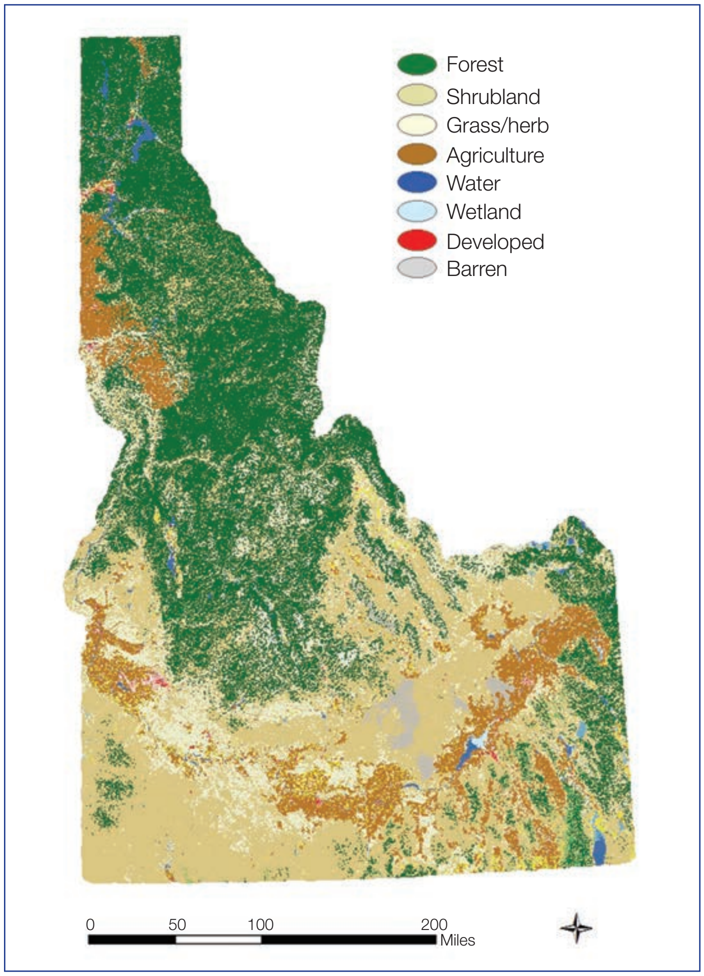

Figure 1. Roughly 40 percent of Idaho is forested. (Source: National landcover dataset, U.S. Geological Survey; map developed by Eva Strand, Assistant Professor, Department of Forest, Rangeland, and Fire Sciences, University of Idaho, Moscow, ID, for this article)

Soils and their underlying parent materials affect Idaho's forest diversity. For example, many of the soils in the central and northern parts of the State have a significant component of volcanic ash, which adds considerably to those soils' ability to retain moisture through the growing season (Garrison-Johnston and others 2007). Soil parent materials are also correlated with forest nutrition on many sites (Moore and Mika 1997).

Commercially harvested coniferous tree species in Idaho include the following:

- Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Mayr] Franco).
- Engelmann spruce (*Picea engelmannii* Parry ex Engelm.).
- Grand fir (*Abies grandis* [Douglas ex D. Don] Lindl.).
- Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. ex S. Watson).
- Ponderosa pine (*Pinus ponderosa* var. p*onderosa* Douglas ex P. Lawson & C. Lawson).
- Subalpine fir (*Abies lasiocarpa* [Hook]) Nutt.).
- Western hemlock (*Tsuga heterophylla* [Raf.] Sarg.).
- Western larch (*Larix occidentalis* Nutt.).
- Western redcedar (*Thuja plicata* Donn ex D. Don).
- Western white pine (*Pinus monticola* Douglas ex D. Don).

Other Idaho tree species are not commonly used for wood products, but have important ecological values. Conifers that fall into this category include white bark pine (*Pinus albicaulis* Engelm.), limber pine (*Pinus flexilis* E. James), alpine larch (*Larix lyallii* Parl.), mountain hemlock (*Tsuga mertensiana* [Bong.] Carrière), and western juniper (*Juniperus occidentalis var. occidentalis* Hook.). Common hardwood species include quaking aspen (*Populus tremuloides* Michx.), black cottonwood, (*Populus trichocarpa* Torr. & A. Gray ex Hook.), and paper birch (*Betula papyrifera* Marshall). Idaho also has dozens of shrub species, including many willow species (*Salix* spp.) (Brunsfeld and Johnson 1985).

Fire has a significant influence on Idaho forests. Many Idaho forests, depending on the site, historically experienced stand-replacement fires every 50 to 500 years and surface fires every 2 to 50 years. Many fire events were a mixture of these two fire types. These fires tended to keep forests in earlier stages of succession (e.g., more pine and larch) than is often seen in many Idaho forests today. The USDA Forest Service national Forest Inventory and Analysis (FIA) program's most recent report on Idaho forests (Witt and others 2012) listed the following top six forest cover type groups in Idaho:

1. Douglas-fir forest cover type.
2. Fir/spruce/mountain hemlock group (includes Engelmann spruce, Engelmann spruce/subalpine fir, grand fir, subalpine fir, and mountain hemlock (*Tsuga mertensiana* [Bong.] Carrière) forest cover types).
3. Lodgepole pine forest cover type.
4. Ponderosa pine forest cover type.
5. Hemlock/sitka spruce (*Picea sitchensis* [Bong.] Carrière) group (includes western hemlock and western redcedar forest cover types).
6. Aspen/birch group (includes quaking aspen, paper birch, and balsam poplar [*Populus balsamifera* L.] forest cover types).

Idaho Forest Ownership

The USDA Forest Service manages the largest part (76 percent) of Idaho forests. Private owners, including forest product companies and family forest owners, hold the second largest portion, roughly 13 percent, of the forest land in Idaho. The State government is the third largest forest owner, with 6 percent (Witt and others 2012). The relative proportions of land in different ownership types vary considerably across the State. Federally managed forests dominate southern Idaho, but progressing farther north into the State's most productive forests, family, State, and industry-owned lands become a larger portion of the mix. For example, in the four northern-most counties of Idaho, 44 percent of the forests are owned by family forest owners (Bundy 1972).

More than 34,000 family forest owners manage timberland in Idaho (Butler 2008). Changes in farming practices (e.g., fewer farmers with livestock) and farm programs, such as the USDA Conservation Reserve Program (CRP), have resulted in former pasture lands or marginal croplands either actively being planted back to trees or passively reverting to forest through old-field succession. Family forest ownerships are also increasing in proportion in some areas of the State, as forest product companies sell their lands and rely on the open market for timber supply.

Idaho also has significant forest land owned and managed by tribal governments. For example, the Coeur d'Alene and Nez Perce tribal governments have forest management staff members in Idaho and active tree planting programs.

Idaho Forest Values and Benefits

Idaho has 340 active forest products manufacturing facilities (IFPC 2013). For many years, harvests from Federal lands provided the largest portion of Idaho's timber volume, but in the past decade, private and State lands provided the largest portion of the harvest. For example, in 2012, private lands and State lands provided 58 and 33 percent of the timber harvested, respectively (Morgan and others 2013).

Forest products are a vital part of Idaho's economy. The total impact in Idaho of converting timber into consumer products (with wood products markets still at a low ebb) is more than $3.2 billion (Morgan and others 2013).

Forests are also critical to water, wildlife, and many other shared values. In addition to their intrinsic values and importance to ecosystem functioning, in 2011, Idaho forests helped support expenditures of $540 million in fishing, $590 million in hunting, $600 million in wildlife viewing, and $350 million in other outdoor recreational activities (Wendland and O'Laughlin 2013).

Forestry Assistance

The USDA Forest Service; the U.S. Department of the Interior, Bureau of Land Management; and other Federal agencies have active tree-planting efforts in Idaho. For the most part, their professional staffs manage their lands, as is the case with forest product companies and some other large forest ownerships. Regarding family forests, a variety of State agencies and nongovernmental organizations support tree planting:

- The Idaho Department of Lands (IDL) manages State-owned lands, provides technical assistance for family forest owners, and administers Idaho's State forest practice laws. These laws focus primarily on reducing fire risk and maintaining forest water quality, but they also include minimum stocking requirements after timber harvests. The IDL employs eight foresters and some seasonal employees who inspect logging jobs for compliance with these State laws and provide assistance to forest owners. Idaho does not currently have any State-level cost share programs, but the IDL works closely with the USDA Natural Resources Conservation Service on federally funded cost share programs that support tree planting, such as the Environmental Quality Incentives Program.
- UI Extension offers multifaceted, research-based information and education programs that help family forest owners, loggers, and foresters manage forests and other natural resources. In addition to providing a variety of workshops, field days, publications, and web offerings for forest owners, UI Extension trains, certifies, and manages Idaho Master Forest Stewards—volunteers who receive 70 hours of training to provide educational assistance to peer forest owners and others interested in forestry.
- Private consulting foresters are also involved in tree planting and other silvicultural practices. Most of this work is with family forest owners, but some consulting foresters also work with forests owned by forest products companies.
- The Idaho Forest Owners Association is the primary organization representing family forest owners in Idaho, both in the State legislature and in a variety of other settings. The association also provides a forum for peer-to-peer learning among forest owners.
- The American Tree Farm System has certified more than 565 forest owners in Idaho.

Idaho Silviculture

Idaho forests are managed for a variety of different benefits, depending on the site and ownership. On many ownerships, fire exclusion and partial harvesting have created denser forests, with a much higher percentage of shade tolerant species (e.g., Douglas-fir, grand fir, western redcedar, and western hemlock) than would have been typically found historically on these sites. This higher density and altered species composition has led to some serious problems with fire risk, insects, and diseases that take advantage of these conditions. For example, root diseases, such as Armillaria (*Armillaria ostoyae*) and laminated root disease (*Phellinus sulphurascens*), and defoliating insects such as tussock moth (*Orgyia pseudotsugata*) and western spruce budworm (*Choristoneura occidentali)* are an issue on many Idaho forests that have become dominated by tree species, such as Douglas-fir and grand fir, most vulnerable to these diseases and insects. On higher elevation forests, many acres of lodgepole pine have been killed during the past 10 years by mountain pine beetle (*Dendroctonus ponderosae* Hopkins, 1902).

Most forest managers' response to these conditions is to reduce stand density and shift species composition to more seral (intermediate) species (figure 2). Habitat types are a land classification system based on the potential climax vegetation for a given site (Cooper and others 1991). The most commonly targeted species for reforestation in Idaho tend to be species that are seral for a site's habitat type and those that will not seed-in naturally. For example, foresters often plant

Figure 2. Western larch is commonly planted in northern Idaho because of its root disease tolerance. (Photo by Chris Schnepf)

ponderosa pine on sites where ponderosa pine or Douglas-fir are climax species; ponderosa pine and western larch on sites where grand fir is the climax species; and progressively more western larch and western white pine, and less ponderosa pine on sites likely to climax in western redcedar or western hemlock. Douglas-fir, lodgepole pine, Engelmann spruce, and western redcedar are occasionally planted, but on most sites, foresters rely on naturally regenerated ingrowth of these and other species.

Tree Improvement

Idaho has a strong tree improvement program, owing in part to white pine blister rust (*Cronartium ribicola* A. Dietr.). Western white pine once dominated moist, midelevation forests in northern Idaho. It is also Idaho's State tree, and was the impetus for the beginning of Idaho's wood products industry, as loggers moved to Idaho for western white pine after depleting eastern white pine (Fins and others 2001). White pine blister rust began to infect Idaho western white pine in the 1920s and quickly invaded white pine sites throughout the State. Fire exclusion, mountain pine beetle, and preemptive harvesting of white pine in the face of blister rust also contributed to the species' decline.

Initial efforts to combat blister rust focused primarily on removing the alternate host (gooseberries and currants; *Ribes* L.) the fungus needs to complete its life cycle. Idaho has at least four *Ribes* species that occur on or near forests. Blister rust fungicides were also attempted. None of these efforts ultimately were very effective at managing the disease.

In the 1950s, USDA Forest Service scientists began noticing trees that seemed to be surviving blister rust, so they began an intensive program to breed blister rust-resistant white pine. That program now produces white pine seedlings that resist blister rust using a variety of mechanisms. White pine still regenerates naturally on many sites in Idaho, but most naturally regenerated trees do not survive. In general, where western white pine is desired, seedlings from the breeding program are planted (figure 3). Breeding efforts to further increase and diversify blister rust resistance are ongoing.

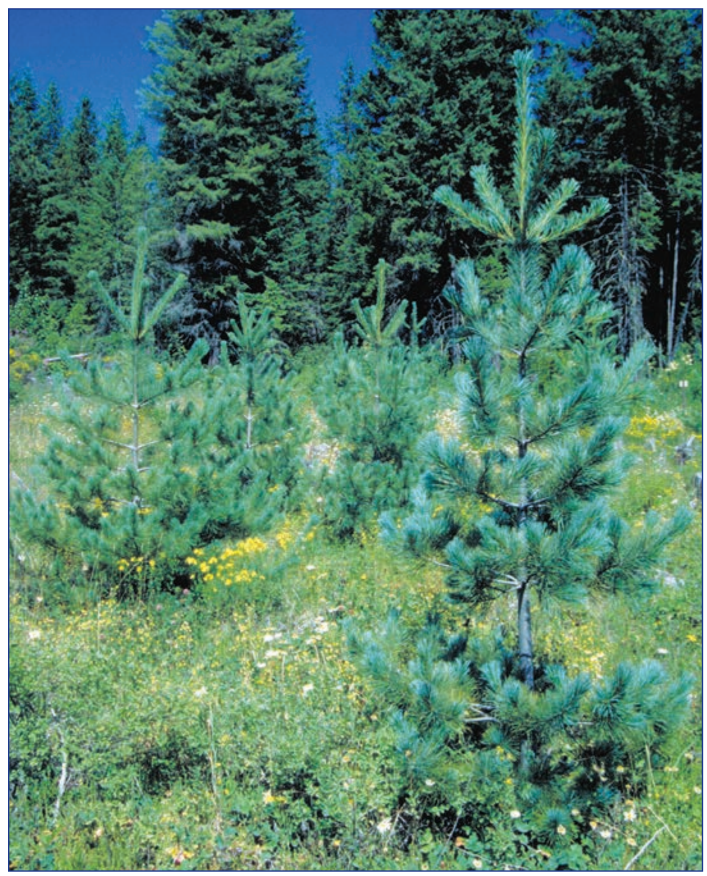

Figure 3. Western white pine is a popular species to plant on moist sites in northern Idaho. (Photo by Chris Schnepf)

An additional fruit of the white pine breeding efforts was the formation of the Inland Empire Tree Improvement Cooperative (IETIC) in 1968. The IETIC is a diverse group of agencies, universities, and forest product companies from northern Idaho, eastern Washington, and western Montana. It is administered through an office at the UI in Moscow. In addition to continuing work on western white pine, the cooperative has breeding programs to produce genetically improved tree seed for ponderosa pine, western larch, Douglas-fir, and lodgepole pine. Since 1974, the IETIC has established more than 120 field tests with more than 1 million seedlings, supported by thousands of parent tree selections in the region's forests. Members have access to IETIC seed and other genetic materials.

Idaho Tree Seedling Nurseries

UI Center for Forest Nursery and Seedling Research

UI began producing seedlings in Moscow in 1909, and since 1926, has functioned as Idaho's defacto State tree nursery. In its early years, the nursery focused exclusively on bareroot seedlings. In 1982, the nursery shifted to container seedling production. The production component of the facility, now known as the Franklin H. Pitkin Forest Nursery, in honor of a former manager of the facility, produces tree and shrub seedlings in a variety of sizes for reforestation, Christmas trees, windbreak plantings, and other conservation efforts. Under the guidance of Dr. David Wenny, the nursery program expanded beyond seedling production under the umbrella of the Center for Seedling and Nursery Research.

In addition to providing seedlings, the center also provides employment and training for students and others interested in tree seedling production (figure 4) and implements research that supports the State's nursery and reforestation industry. The center operates in consultation with an advisory committee that includes representatives of Idaho's nursery industry. In February 2013, the center received a $3.3 million endowment to establish a new classroom and support graduate and faculty research.

Conservation Districts

Many Idaho soil and water conservation districts, particularly in the northern end of the State, sell tree seedlings for reforestation and conservation plantings. Typically, tree seedlings are grown by contract with private nurseries, then seedlings are sold and distributed through local conservation district offices.

Figure 4. Idaho Master Forest Stewards learning about seedling production at the University of Idaho Center for Seedling and Nursery Research. (Photo by Chris Schnepf)

The USDA Forest Service Coeur d'Alene and Lucky Peak Nurseries

The USDA Forest Service nursery in Coeur d'Alene (figure 5) grows a variety of nursery stock types for planting on publicly owned lands in the region. Most of these are conifer tree seedlings for reforestation, but the nursery also grows a variety of other native plants for habitat restoration efforts (e.g., grass and sedge plugs and rooted cuttings). The nursery can produce more than 16 million seedlings from 130 ac (53 ha) of irrigated seedbeds and an additional 4 million container seedlings in 25 controlled-environment greenhouses. The nursery also cleans, tests, and stores seeds, and it provides seedling quality testing.

Located near Boise, the Lucky Peak Nursery has produced seedlings since 1959. It stores seed and grows seedlings for national forests and other publicly owned lands in the Intermountain West Region. One of its specialties is producing bareroot desert shrubs, such as sagebrush (*Artemisia* L.) and bitterbrush (*Purshia tridentata* [Pursh] DC.). Annual production ranges from 2 to 6 million trees on 60 ac (24 ha) of land. The nursery produces both container and bareroot seedlings.

Figure 5. The USDA Forest Service nursery in Coeur d'Alene grows a variety of nursery stock types for planting on publicly owned lands in the region. (Photo by Chris Schnepf)

Private Tree Seedling Nurseries in and Near Idaho

Idaho has a handful of private nurseries that grow seedlings for reforestation or conservation plantings. Idaho also has a sizeable woody ornamental nursery industry, located primarily in the northern end of the State, that grows a variety of trees, shrubs, and groundcovers for the retail and wholesale nursery trade.

Tree Planting Challenges in Idaho

Idaho's varied topography, climate, and soils likewise bring varied challenges to tree planting in the State:

Seedling Moisture Stress

The lack of available soil moisture limits growth in most Idaho forests. Methods used to mitigate this condition include—

- Prescribed burning, scarification, scalping, and herbicide treatments to reduce competing vegetation
- Robust seedlings with a good shoot-to-root ratio.
- Microsite shade, primarily using materials on site, such as pieces of logs, or stumps (figure 6). Stumps are usually avoided on planting sites with a recent history of aggravated root disease, however. Shingles or shade cards are sometimes used on especially difficult sites.

Figure 6. Microsite shade is commonly used to reduce seedling moisture stress in Idaho. (Photo by Chris Schnepf)

Rodents

Pocket gophers *(Thomomys talpoides* [Richardson 1828]) can cause significant seedling mortality in Idaho reforestation efforts, especially where the habitat is ideal for this rodent. Meadow voles (*Microtus pennsylvanicus* [Ord 1815]) can also cause notable seedling mortality, particularly on afforestation efforts on former farm fields. Toxicants placed underground are the most common method of dealing with pocket gophers. Porcupines (*Erethizon dorsatus* [Linnaeus 1758]) can be an issue on some sites, but in Idaho, they are more commonly a problem on sapling or larger trees.

Deer, Elk, and Moose

White tail deer (*Odocoileus virginianus* [Zimmermann 1780]), mule deer *(O. hemionus* [Rafinesque 1817]), elk (*Cervus elaphus* [Linnaeus 1758]), and moose *(Alces americanus* [Clinton 1822]) all frequently browse on Idaho tree seedlings, especially on sites that coincide with winter range for these animals. The most common methods used to help seedlings survive browse damage are rigid plastic mesh tubes (figure 7) and repellents. As trees grow older, individual saplings are occasionally damaged by ungulates rubbing the velvet from their antlers, though sometimes trees survive this activity. Western red-cedar and hardwood species, such as aspen, often cannot be successfully established without protection from ungulates.

Figure 7. Animal damage protection can be critical in some Idaho reforestation efforts. (Photo by Chris Schnepf)

White Pine Blister Rust

While planting western white pine from the IETIC breeding program has brought considerable progress in reestablishing this valued species, blister rust must be monitored in white pine plantations (Schnepf and Schwandt 2006). Blister rust has its greatest effect on young trees because they have more green branches close to the ground, where higher humidity increases infection risk. While blister rust-resistant seedlings have a good chance of surviving the fungus, resistance varies considerably by site. Pruning the bottom 10 feet of young trees (figure 8) can reduce blister rust mortality of naturally regenerated western white pine by 50 percent (Schwandt and others 1994). Even blister rust-resistant trees increasingly are being pruned to enhance survival, especially on sites with a high blister rust hazard (e.g., high humidity and *Ribes* density).

Figure 8. Pruning western white pine can cut blister rust mortality in half. (Photo by Chris Schnepf)

The Future

A variety of challenges and opportunities are on the horizon for tree planting in Idaho. It is not yet clear what climate change may bring to local sites, but landowners and managers are discussing potential climate scenarios and management responses. Species recommendations and seed transfer zones have not yet been revised in anticipation of climate change.

With lumber mills' growing capacity to use smaller diameter trees (figure 9), the incentive to plant trees has increased because planting costs are not held as long. Better sites in northern Idaho can produce small diameter saw logs in as little as 25 years. In addition, a great deal of research is underway in the region regarding new uses of forest biomass, both with native species and with hybrid poplars. If these markets develop, they will also provide an opportunity to use trees from precommercial thinning activities, or even plant trees with biomass as the primary end product.

Figure 9. Inland Northwest lumber mills' growing capacity to use smaller diameter logs increases the incentive to plant trees. (Photo by Chris Schnepf)

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Germination Trials for Asian and North American Ash Species

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Abstract

North American ash trees (genus *Fraxinus*) have been affected by the emerald ash borer (*Agrilus planipennis* Fairmaire; EAB), an aggressive, invasive insect native to southeastern Asia; native Asian ash species are comparatively resistant to this phloem-feeding insect. Little research exists on optimal growing conditions for germinating seeds from various ash species, aside from those with the widest natural ranges. The objective for this research was to evaluate the effects of temperature, photoperiod, and seed scarification on germination of nine ash species. Germination rates and percentages were evaluated under three test conditions (controlled environment, agar-based solid medium, and potting mix). Chinese and green ash seeds germinated the best, while black, blue, common, and Manchurian ash seeds had the lowest germination rates across all three tests. Optimized germination techniques for ash will enable research into the EAB resistance mechanism of some ash species. In addition, North American nurseries may be able to use the information presented here to more efficiently grow Asian or other ash species that may be resistant to EAB.

Introduction

Ash trees (genus *Fraxinus*) are important in North America. These trees are fundamental aesthetic elements of nearly every city and suburban landscape, have high stumpage value, and are an important commercial lumber and pulp species for the furniture and paper- and tool-making industries. In addition, ash trees serve as significant Native American cultural resources and play an integral part in the ecology of North America (Cappaert and others 2005). The ash-tree industry in North America is threatened by a recently introduced invasive species: the emerald ash borer (*Agrilus planipennis* Fairmaire; EAB; MacFarlane and Meyer 2005; Poland and McCullough 2006). EAB larvae feed on phloem of ash trees, ultimately killing the tree by restricting vascular transport (figure 1). Because of the introduction of the EAB in North America, most nurseries in the United States, even those in the West, where EAB has yet to arrive, have stopped growing *Fraxinus* species. As a result, ash seedlings are increasingly unavailable to researchers interested in studying ash (especially in the context of EAB), so they must grow their own (figure 2). Little information has been published on optimal conditions for germination of Asian and European ash species. What limited research that has been done has focused on seed cutting or embryo-rescue treatments (both of which are time consuming) in the most common North American ash species, such as green (*Fraxinus pennsylvanica* Marshall), white (*F. americana* L.), black (*F. nigra* Marshall), and the European common ash (*F. excelsior* L.) (Steinbauer 1937; Villiers and Wareing 1964, 1965; McBride and Dickson 1972; Bonner 1975; Marshall 1981; Stinemetz and Roberts 1984;

Figure 1. Emerald ash borer larvae galleries under the bark of a mature white ash tree in Fort Wayne, IN, May 2009. (Photo by Darla French)

Figure 2. Three-year-old white ash trees that the authors grew from seed and maintained in the greenhouse. (Photo by Darla French)

Vandewalle 1985; Piotto 1994; Preece and others 1995; Piotto and Piccini 1998; Ashley 2002; Raquin and others 2002; Ashley and Preece 2004, 2009).

The objective for this research was to evaluate varying environmental conditions and seed treatments on germination of nine ash species.

Materials and Methods

Seed Source and Preparation

Nine ash species were included in this study (table 1). All seeds were stored in sealed containers over a desiccant at 40 °F (4 °C) in the dark until testing (up to 12 months). Seeds of all species were germinated under three different test conditions: potting mix; a controlled environment; and an agar-based, solid medium.

Before the germination tests, the pericarps were removed from the seeds, except for some of those used in the potting-mix test, in which pericarps were removed for two treatments and left intact for the third. To remove pericarps, seeds were soaked in tap water at room temperature for several hours to soften tissue and facilitate removal. Before the tests on an agar-based, solid medium and potting mix, seeds were imbibed for 20 minutes using 0.3M NaOH, followed by surface sterilization at two concentrations of calcium hypochlorite [$Ca(ClO)_2$] solution, each with 0.01 percent Tween® 20 (Sigma-Aldrich, St. Louis, MO) according to a protocol described by Raquin and others (2002). At the end of sterilization, seeds became white and translucent, and embryos were clearly visible. Seeds damaged by insects and those without embryos or showing necrosis were discarded. Seeds were then subjected to the tests described below. In all tests, seeds were considered germinated when both a radicle hook and cotyledons were apparent (figure 3).

Table 1. Ash (*Fraxinus*) species and seed sources used in this study. Seeds for all tests were obtained in 2009 and 2010 from Sheffield's Seed Co., Inc. (Locke, NY), or Lawyer Nursery (Plains, MT). Lot numbers available upon request.

Species	Scientific name	Seed source
Black ash[a]	*F. nigra* Marshall	Ontario, Canada
Blue ash[a]	*F. quadrangulata* Michx.	Indiana, United States
Chinese ash[a]	*F. chinensis* Roxb.	Beijing, China
European common ash[a]	*F. excelsior* L.	Poland
Flowering ash	*F. ornus* L.	Hungary
Green ash[a, b]	*F. pennsylvanica* Marshall	South Dakota, United States[a] Pennsylvania, United States[b]
Manchurian ash[a, b]	*F. mandschurica* Rupr.	Beijing, China
Pumpkin ash[a]	*F. profunda* Bush	Louisiana, United States
White ash[a, b]	*F. americana* L.	North Dakota, United States

[a] Seed from Sheffield's Seed Co., Inc. (Locke, NY).
[b] Seed from Lawyer Nursery (Plains, MT).

Figure 3. Example of a germinated Chinese ash seedling exhibiting a radicle hook and the presence of cotyledons. (Photo by Darla French)

Controlled-Environment Test

For the controlled-environment test, incubators were set at four constant temperature treatments: 50, 59, 68, and 77 °F (10, 15, 20, and 25 °C, respectively) and a fifth treatment consisted of alternating 59 °F in the dark and 77 °F in the light (15 and 25 °C, respectively). At each temperature treatment, 8-h and 16-h photoperiods were used for a total of 10 treatments. There were three replicates for each temperature/photoperiod treatment. One hundred intact seeds per treatment replicate per species were placed on blotter paper in clear 6-oz (177-ml), 3.5-in (89.0-mm) diameter plastic jars (Parkway Plastics, Inc., Piscataway, NJ) and were wetted with ~0.2 oz (~5.0 ml) tap water (figure 4). Seeds were neither imbibed nor sterilized before the test. Containers were left in the incubators for 30 days, with germination recorded every 5 days. Rewetting of blotter paper with tap water was performed periodically during the 30 days.

Figure 4. Chinese ash seeds and seedlings 20 days after sowing in the controlled-environment experiment. (Photo by Darla French)

Agar-Based, Solid-Medium Test

For the agar-based, solid-medium test, imbibed, sterilized seeds were plated on Murashige and Skoog (MS) media (Murashige and Skoog 1962) containing 3 percent sucrose. Seeds were either left intact, or were cut to remove 0.2 in (0.5 cm) from cotyledonary ends of seeds as described by Ashley and Preece (2004). Three replications were made of at least 100 seeds per species for each of the two treatments. Plated seeds were left at room temperature under 40W Sylvania Gro-Lux lamps for 60 days (16-h photoperiod), with germination recorded every 5 days (figure 5).

Figure 5. Green ash seedlings 30 days after sowing on agar-based solid medium. (Photo by Darla French)

Potting-Mix Test

For the potting-mix tests, sterilized seeds were planted in standard 36-cell seed trays (overall dimensions: 11 by 21 in [28 by 54 cm]; volume of each cell: ~3 in^3 [49 cm^3]) in Premier® Pro-Mix® PGX Grower Mix (Premier Horticulture, Ltd., Quakertown, PA), a high-porosity, peat-based germination and growing medium optimized for seedling production in plug systems (figure 6). Seed were sown at a depth of 0.2 to 0.4 in (0.5 to 1.0 cm) as recommended by the supplier. As with those tested in the agar-based, solid medium, seeds were either left intact, or they were cut as described by Ashley and Preece (2004). In a third treatment, seeds without the pericarp removed were sown into the potting mix. Three replications were made of at least 100 seeds per species for each treatment. Seeds were left under a combination of 600W high-intensity discharge lamps; high-pressure sodium lamps (PARsource Lighting Solutions, Petaluma, CA); and 60W cool white, energy-efficient fluorescent lamps (4,100 K [color temperature], 6,150 initial lumens; Grainger, Inc.. Lake Forest, IL) for 60 days (16-h photoperiod), with germination recorded every 5 days. Seed trays were watered regularly to maintain adequate moisture in the potting mix.

Statistical Analyses

Statistical comparisons were made between treatments for each experiment using one-way analysis of variance (ANOVA) based on means of three replicates per test.

Figure 6. Green ash seedlings 20 days after sowing on potting mix medium. (Photo by Darla French)

Results

Controlled-Environment Test

ANOVA tests were used to analyze germination rates after 30 days in the controlled-environment experiment by species, temperature, and photoperiod (table 2). Chinese and green ash seeds had higher germination rates as temperature increased, regardless of photoperiod. In addition, both Chinese and green ash seeds had greater germination under the shorter photoperiod than seeds at the same temperature under the longer photoperiod. Pumpkin ash only germinated at 68 °F (20 °C) under a short photoperiod but did not germinate under any other conditions. Black, common, and Manchurian ash seeds failed to germinate under any condition, suggesting their stratification needs and/or required germination conditions were not met.

Agar-Based, Solid-Medium Test

For all species on the MS medium, germination rates tended to be more rapid and higher for seeds in the "cut-seed" treatment than those in the "intact-seed" treatment (table 3); however, flowering ash was the only species for which a statistically significant difference occurred between treatment groups ($p = 0.0108$).

Table 2. Germination percentages for five species of ash seeds germinated on wetted filter paper in incubators over 30 days under five temperature regimes and two photoperiods. Black, blue, common, and Manchurian ash seeds were also tested but did not germinate under any temperature/photoperiod regime. Species names followed by the same letter did not differ significantly in overall germination after 30 days at alpha = 0.05. No statistically significant differences were observed among temperature treatments. The 8-h photoperiod resulted in significantly higher overall germination ($p = 0.0051$) than the 16-h photoperiod. No statistically significant interactions occurred among species, temperature, or photoperiod.

Ash species (significance)	Days after sowing	10 °C		15 °C		20 °C		25 °C		15–25 °C	
		8 h	16 h	8 h	16 h	8 h	16 h	8 h	16 h	8 h	16 h
Chinese (A)	10	1	0	34	0	55	3	15	14	81	0
	20	1	0	37	2	59	19	26	28	93	2
	30	1	0	37	6	59	20	26	30	93	2
Flowering (B)	10	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	2	0	4	0	0	37	0
	30	0	17	1	15	0	4	0	0	43	0
Green (A, B)	10	0	0	6	0	29	1	6	3	85	0
	20	0	0	24	1	34	8	6	7	89	0
	30	1	0	24	1	34	9	6	7	89	0
Pumpkin (B)	10	0	0	0	0	39	0	0	0	0	0
	20	0	0	0	0	40	0	0	0	0	0
	30	0	0	0	0	40	0	0	0	0	0
White (B)	10	0	0	0	0	0	0	0	0	0	0
	20	0	0	2	0	0	0	0	0	5	0
	30	9	0	3	0	0	0	0	0	5	0

Table 3. Percent germination for eight species of ash seeds on agar-based medium. Seeds were either cut or left intact. Blue ash seeds were also tested, but they did not germinate under either seed treatment. Within a species, means did not differ significantly among treatments with the exception of flowering ash.

Days after sowing	Black		Chinese		Common		Flowering		Green		Manchurian		Pumpkin		White	
	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact	Seed cut	Seed intact
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	4	2	0	0	0	0	0	0	0	0	0	0	0	0
10	1	0	12	48	0	6	0	0	42	8	0	0	0	0	0	2
15	3	0	21	48	0	6	0	0	49	8	0	0	0	0	0	2
20	5	0	24	52	0	6	0	0	58	21	1	0	0	0	2	2
25	5	5	26	56	1	6	11	4	62	35	3	0	2	0	3	2
30	5	5	26	56	1	6	18	5	65	35	9	0	6	0	3	2
35	8	5	26	56	5	6	24	5	66	35	11	0	11	0	8	2
40	8	5	27	56	5	6	37	8	66	36	12	1	12	0	8	2
45	8	5	44	56	10	9	54	16	66	37	13	1	19	0	9	2
50	9	5	49	56	10	9	55	17	66	37	18	1	20	0	12	2
55	11	5	57	56	10	9	55	17	66	52	22	1	20	0	12	2
60	11	5	57	56	10	9	55	18	66	52	27	1	20	0	12	2

Potting-Mix Test

When sown into potting mix, only one-half of the species germinated (table 4). Within those, no seeds in the "pericarp removed" treatment germinated. Of the other two treatments, more seeds germinated if they were left intact than if they were cut, though none of those differences were statistically significant, likely because of the high variability between replicates.

Discussion

Seeds require specific environmental conditions to emerge from dormancy. The requirements for germination are species-specific and include variables such as temperature, moisture, and photoperiod. Chinese and green ash seeds germinated the best across all three tests and all treatment levels; unless more optimal conditions are found for seeds of the remaining species of ash, future research will likely focus on these two species. While tetrazolium chloride tests were not completed to test seed viability, it can be inferred that the seeds used in this study were alive, because all species showed at least some level of germination in the agar-based, solid-medium test.

Under the conditions tested, flowering, pumpkin, and white ash seeds were moderately difficult to germinate. In the case of these three species, dormancy may involve chemical inhibitors (Villiers and Wareing 1964, 1965; McBride and Dickson 1972) that are not present in Chinese and green ash seeds. Ashley and Preece (2004) suggest that white ash seeds have a much deeper, more complex dormancy compared with Chinese and green ash seeds.

Table 4. Percentages of seeds from five species of ash germinated on a peat-based potting mix in a greenhouse. Seeds were either cut or left intact; intact seeds either had the pericarp intact or removed. Seeds with pericarps did not germinate. Between the other two treatments, the germination means were not statistically different for any species.

Days after sowing	Chinese			Flowering			Green			Pumpkin			White		
	Seed cut	Seed intact without pericarp	Seed intact with pericarp	Seed cut	Seed intact without pericarp	Seed intact with pericarp	Seed cut	Seed intact without pericarp	Seed intact with pericarp	Seed cut	Seed intact without pericarp	Seed intact with pericarp	Seed cut	Seed intact without pericarp	Seed intact with pericarp
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
10	3	6	0	0	0	0	6	3	0	0	0	0	1	0	0
15	11	14	0	0	0	0	11	7	0	0	0	0	1	0	0
20	17	16	0	0	0	0	13	15	0	0	0	0	1	1	0
25	18	19	0	0	0	0	16	25	0	0	1	0	1	1	0
30	18	20	0	0	1	0	17	31	0	0	11	0	2	1	0
35	18	20	0	0	1	0	17	32	0	0	11	0	2	1	0
40	18	20	0	0	1	0	17	32	0	0	11	0	2	1	0
45	18	21	0	0	1	0	17	32	0	0	12	0	2	1	0
50	19	21	0	0	1	0	17	33	0	0	15	0	2	1	0
55	27	29	0	0	1	0	17	33	0	0	15	0	2	1	0
60	36	41	0	0	1	0	17	33	0	0	15	0	2	1	0

Black, blue, common, and Manchurian ash seeds had the lowest germination rates across tests, which may indicate that their embryos were immature. Some evidence suggests that low germination of black and common ash may be because of immature embryos (Steinbauer 1937; Villiers and Wareing 1964; Vanstone and LaCroix 1975; Wagner 1996). Because Manchurian ash seeds appear to have similar seed and embryo morphology to those in black and common ash, their lower germination may also be related to physiologically immature embryos.

Embryo excision and culture have been used to overcome dormancy in many cases, such as when a chemical inhibitor is present, when the seed coat is impenetrable to the growing embryo, or when seeds require complex stratification to overcome dormancy (Arrillaga and others 1992). Embryo culture, however, is often a difficult, labor-intensive process that requires extreme care to avoid damaging the embryo, and our experience has shown that, for most species of ash, the percentage of embryos that survived the excision process is very low (data not shown). Therefore, manual embryo excision is not a recommended method for increasing ash seed germination rates. In this study, seed cutting was a quicker way to increase germination rates without excessively damaging embryos, although for species with immature embryos, including black (Vanstone and LaCroix 1975) and Manchurian ash, even seed cutting may not significantly improve germination, as indicated by the low germination rates for these species in this study.

Hormonal treatments are often used to overcome seed dormancy. In the case of common ash, Lewandowska and Szczotka (1992) showed that addition of kinetin or gibberellin before cold stratification stimulated the breaking of dormancy but lowered overall germination rates, as compared with a control group. Determining which hormones, at what rate, and in conjunction with what temperature or humidity, however, can be a time-consuming process. Still, it may be a worthwhile undertaking for seeds from some species, such as flowering, pumpkin, and white ash, which showed low overall germination rates, even with seed cutting.

The method of Ashley and Preece (2004) for removing the cotyledonary tip of the ash seed to promote germination is much more efficient than embryo culture or hormone treatments at overcoming dormancy for most species of ash. Nurseries and researchers may be able to use the information obtained here as a first step toward identifying the quickest and most optimal germination conditions for a variety of *Fraxinus* species. Based on our results, photoperiod seems to have a greater effect than temperature on the germination of Chinese, green, and pumpkin ash seeds. This environmental effect may be because of differing evolutionary adaptations for climate, or it may suggest a deeper interaction between photoperiod and temperature on germination rate, as is seen in eastern hemlock (*Tsuga canadensis* L.; Stearns and Olson 1958). Our results suggest that the ash seed pericarp, while protecting the seed from environmental conditions, also serves as a physical barrier to germination (possibly also as a chemical barrier; Thapliyal and Nautiyal 1989). Further, an intact seed coat (i.e., one that has not received any cutting treatment) may prevent endosperm degradation products from leaching out, as may happen with cut seeds placed in potting mix or soil. On solid media, perhaps nutrients from the endosperm are less likely to leach from the incision because the seed sits only on top of the media, rather than being embedded in moist potting mix, as was done here.

The slower, lower overall germination rates of black, blue, common, flowering, Manchurian, pumpkin, and white ash as compared with Chinese and green ash (table 3) may suggest that the denser, thicker seed coats and slightly different seed shape we observed for seeds of these species act as germination barriers. As suggested by Ashley and Preece (2004), the cutting treatment may serve a dual purpose in enhancing germination rates: first, by removing a physical barrier to the emergence of radicles and cotyledons and, second, by allowing for a more efficient gas exchange, thereby promoting the germinative process (Villiers and Wareing 1965).

We recommend that a better procedure for surface sterilization and imbibition of ash seeds be developed. Microbial contamination was a major limitation in these germination experiments. While the procedure for preparing seeds for plating or planting included two progressively more stringent steps for sterilization (Raquin and others 2002) during initial imbibition, mold and fungi were major problems in both the incubator and agar-based, solid-medium tests. This undesirable effect may be because of incomplete surface sterilization (Singh and others 1992). A more reliable procedure could be developed by including additional sterilization steps or reagents. Tetrazolium chloride tests should also be implemented to ensure that seedlot viability is as expected. In addition, humidity control could be incorporated into future trials to further evaluate optimum conditions for germinating ash seeds.

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Nursery Lifter Operation Affects Root Growth Potential of Pine Seedlings

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Abstract

Root damage to seedlings is inherent in a bareroot lifting operation. Full-bed lifters lift all seedling drills across a nursery bed at one time, whereas two-row lifters selectively lift any two seedling drills across a nursery bed but require multiple passes to lift all the seedlings. To determine the extent of root damage among lifting methods, we compared roots of seedlings lifted with a two-row or a full-bed lifter, each operated at the normal calibrated speed and a faster noncalibrated speed, to hand-lifted seedlings at three nurseries in the Southern United States. When root growth potential (RGP) and root morphology were used to evaluate lifter speed for the full-bed lifter, two of the three nurseries had greater RGP or root morphology measurements at a faster tractor speed. The use of two-row seedling lifters, which travel four to six times faster than full-bed lifters, resulted in significantly more root injury than hand-lifted seedlings. No difference existed in root biomass or root weight ratio measurements with nursery treatments. If nursery staff use ocular comparisons of seedling roots to evaluate lifter efficiency, careful attention needs to be given to the presence of fine root tips, mycorrhizae, and damage to root cortex on lateral roots that could cause a reduction in RGP.

Introduction

A priority for bareroot nursery managers during the lifting process is to minimize seedling damage caused by lifting equipment. Before 1934, all seedlings were lifted by hand using a shovel, but beginning in 1935, simple lifting blades were developed to cut the taproot and loosen the soil, thereby enabling crews to manually remove seedlings from nursery beds (May 1984). In 1958, the Agricultural Engineering Department at the University of Georgia developed the first mechanical harvester capable of loosening and lifting a full bed of eight seedling rows (Darby 1962). This machine became the prototype of other full-bed (eight-row) lifters as well as partial-bed lifters that are now used in the Southern United States (May 1984). Partial bed lifters (e.g., Mathis®) caught on in seedling production because they were less expensive (Sampson 1972) and operated at a higher ground speed than full-bed lifters, allowing for similar numbers of lifted seedlings per day (Sampson 1972, Black 1976). Unlike full-bed lifters, partial-bed lifters require multiple passes over the nursery bed to lift all seedlings.

Seedling lifters are pulled by a tractor and powered by a power take-off-driven hydraulic pump. As the lifter is pulled down the nursery bed, pairs of counter-running pickup belts are lowered to grab seedling stems at the ground line and gently lift individual rows (drills) of seedlings out of the soil. A full-bed lifter would typically have eight pairs of belts, one pair for each seedling drill. Before the belts lift the seedlings, the taproots are generally cut to approximately 6.0 in (15.2 cm) and the nursery bed loosened using either the lifter blade on the full-bed lifter or in a separate operation. One- and two-row lifters are not equipped with a lifter blade and require a separate operation to cut the taproot and loosen the soil nursery bed before lifting seedlings. Root shakers loosen excess soil as seedlings move up the belts. When the seedlings reach the end of the belts, they are directed either to seedling bins for shed packing operations or to personnel who place the seedlings directly into bags for field packing.

In contrast to mechanical lifters, some nurseries in the Southern United States continue to hand-lift their entire seedling crop. Nurseries use this method because mechanical lifters are more difficult to use in fine texture (heavy soils) and the cost of a mechanical lifter may not be economical based upon the number of seedlings grown. In this case, the seedling beds are undercut, the root systems are shaken and loosened by a tractor-pulled machine, and then the seedlings are hand-lifted and placed into either tubs or crates.

In the Southern United States, nearly all nurseries lateral-prune their seedlings within the nursery beds at least one time in early to middle fall. The lateral pruning severs the lateral roots between the seedling drills, which facilitates machine lifting. Nurseries also undercut the nursery beds at least one time before lifting regardless of whether the lifter blade on the full-bed lifter is used.

Nursery managers at bareroot nurseries take precautions to minimize seedling injury during the lifting and shipping process. At the beginning of the lifting season, nurseries generally follow a three-step calibration process. First, the lifter belts are adjusted so that their speed is slightly faster than the tractor ground speed. If the belt speed is too fast, seedlings are snatched from the ground causing root injury (figure 1) or injury to the root collar region. When the belt speed is too slow, seedlings are not separated coming up the belt, which leaves the roots susceptible to tearing during the packing process. The normal calibrated tractor speed used by a nursery is determined based on soil texture. Second, the root shakers are adjusted so as to leave some soil on the roots to prevent drying out. Too much soil can cause further root injury in handling. The third step in seedling lifter calibration involves comparing the root mass of seedlings lifted with a shovel from an adjacent area to seedlings from the lifter. Individual seedlings are examined for root biomass and the presence of fine root tips, mycorrhizae, or damage to lateral roots or to the root collar region. It is common for individual nurseries to make additional modifications to their seedling lifters based on their soil texture in an effort to maintain seedling quality.

In addition, nurseries try to minimize root exposure after lifting by spraying roots with acrylic-based gels, storing seedlings in a cooler, and shipping in refrigerated trucks. Examining the nursery bed behind any lifter today will reveal numerous fine roots remaining in the soil. Rowan (1987) reported that lifting bareroot seedlings from nursery beds can remove 35 to 77 percent of small roots from seedlings. South and Stumpff (1990) reported that a loss of 22 percent of the "short roots" and a few of the higher order "long lateral" roots reduced root growth potential (RGP) by 50 percent. The stripping of roots by machine lifters can increase seedling mortality after outplanting up to 50 percent (Langdon 1954; Wakeley 1965; Barnard and others 1980; Xydias 1982; Rowan 1987; Reynolds and others 2002). Summarizing these studies, South and Cary (2001) suggest that one- or two-row lifters had greater seedling mortality compared with full-bed lifters. Because the type and speed of lifter are two factors that can be adjusted, the purpose of this study was to compare RGP, root biomass, and root morphology of pine seedlings lifted with either a two-row or full-bed lifter operated at two different speeds.

Methods

Three bareroot nurseries located within the Coastal Plain region of the Southern United States were chosen for this study (figure 2, table 1). At Nursery A, slash pine (*Pinus elliottii* Engelm.) seedlings were lifted on December 15, 2010 using a Mathis® two-row lifter (figures 3 and 4) and a Love® full-bed lifter (figure 5). At Nurseries B and C, loblolly pine (*P. taeda* L.) seedlings were lifted on February 9 and February 23, 2011, respectively, using a Love® full-bed lifter. The Mathis® two-row lifter can be adjusted to lift any two seedling drills within a nursery bed (figure 6) whereas the Love® full-bed lifter removes all drills across the seedling bed (figure 7). The Love® full-bed lifter also has a seedling lifter blade that can be raised or lowered to assist in loosening soil and seedlings during operation (figure 8); this blade was used only at Nursery C. At each nursery, the lifter(s) were operated at two speeds: the normal speed at which the lifter was calibrated and a faster speed (table 2).

At each nursery, four sections (replications) of a bed row (approximately 80 ft [24 m]) were selected for the study. Within each 80 ft (24 m) section, the lifter(s) were operated at the

Figure 1. Example of seedling lifter damage to lateral roots. (Photo by Tom E. Starkey)

Figure 2. Example of southern coastal plain bareroot nursery. (Photo by Tom E. Starkey)

Table 1. Nursery, species, seedling density, date lifted, and nursery soil characteristics of the three nurseries included in this study.

Nursery	Pine species	Seedling density ft² (m²)	Date lifted	Soil moisture (%)	Percent		
					Sand	Silt	Clay
A	Slash	21 (233)	12/15/2010	7.1	84	9	7
B	Loblolly	23 (255)	2/9/2011	10.1	83	9	8
C	Loblolly	21 (233)	2/23/2011	6.4	74	15	11

Figure 3. Mathis® two-row seedling lifter. (Photo by Tom E. Starkey)

Figure 5. Lifter belts for the Love® full-bed seedling lifter. Each of the two adjacent belts lifts one row of seedlings. (Photo by Tom E. Starkey)

Figure 4. One row of lifter belts for the Mathis® two-row lifter. (Photo by Tom E. Starkey)

Figure 6. Mathis® two-row lifter adjusted to lift seedling drills 3 and 6. (Photo by Tom E. Starkey)

two different speeds. This practice allowed for the collection of approximately 50 seedlings for each lifting speed in each replication. In addition, approximately 50 seedlings, designated as control seedlings, were hand lifted using a shovel from the third seedling drill in each 80-ft (24-m) plot at each nursery before any mechanical lifting. Hand-lifted seedlings were chosen as our control, because nurseries generally use these seedlings to evaluate the effectiveness of seedling lifter calibration.

Replications for 25 seedlings per treatment were measured for root collar diameter (RCD), height, shoot and root biomass, and root weight ratio (RWR, defined as the root weight divided by total seedling weight). The roots from 10 of these seedlings per treatment replication were selected before drying for root morphology measurements using WinRhizo computer software using a flatbed scanner (Regents Instruments Inc., Quebec, Canada). Root morphology data included root volume, root length, number of root tips, and number of

Figure 7. Seedling bed after seedlings are removed using Love® full-bed lifter. (Photo by Tom E. Starkey)

Figure 8. Love® full-bed lifter with seedling lifter bar in raised (unused) position (top) and in lowered (in use) position (bottom). (Photo by Tom E. Starkey [top] and Ben Whitaker, Auburn University [bottom])

Table 2. Lifter type and lifting speed used to remove seedlings from nursery beds.

Nursery	Lifter	Lifter blade used?	Normal speed mph (kph)	Fast speed mph (kph)
A	Mathis® two-row	NA	1.50 (2.4)	2.00 (3.22)
A	Love® full-bed	No	0.25 (0.40)	0.50 (0.81)
B	Love® full-bed	No	0.33 (0.53)	0.39 (0.63)
C	Love® full-bed	Yes	0.50 (0.81)	0.70 (1.13)

NA = not applicable

root forks (a rough estimate of mycorrhizae). Forty seedlings (five seedlings per treatment by eight replications) were placed in aquariums (figure 9) with aerated water for 30 days then evaluated for RGP by counting the number of white root tips that are greater than 0.5 cm (0.2 in) (Palmer and Holen 1986; figure 10). Analysis of variance was performed using the PROC GLM function to test for treatment differences at an alpha level of 0.05. Treatment means were separated using Duncan's Multiple Range Test (SAS Institute 2003).

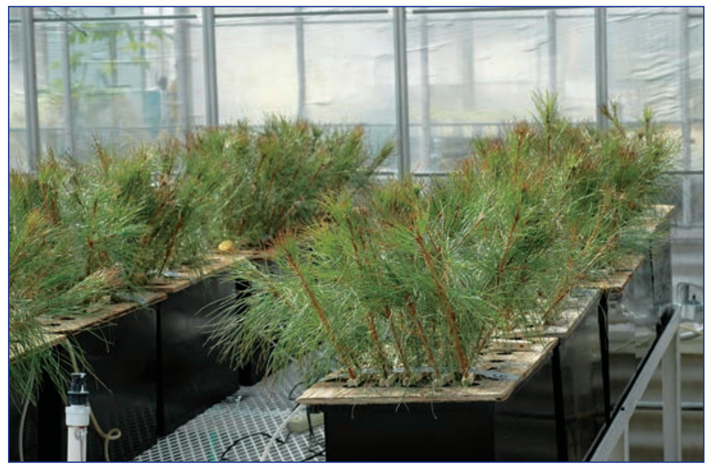

Figure 9. Aquariums used for root growth potential. (Photo by Tom E. Starkey)

Results

Nursery A

Lifter type or speed had no effect on seedling height, root biomass, or shoot biomass (data not shown). No significant difference existed for RWR between lifters or lifter speed (table 3). Seedlings lifted by the Love® full-bed lifter averaged 6 percent larger RCD than those collected from the Mathis® two-row lifter (data not shown). The full-bed lifter operated at the faster speed had 85 percent more white root tips than the normal speed. The speed of the two-row Mathis® lifter had no effect on RGP when comparing white root tips. The hand-lifted controls and the Love® full-bed fast speed

Figure 10. Slash pine roots ready to have root growth potential (RGP) white root tips counted (left) and white root tips being counted for RGP determination (right). (Photos by Paul Jackson, Louisiana Tech University [left] and Tom E. Starkey [right])

Table 3. A comparison of treatment means for root characteristics and root weight ratio at each nursery. Means within a column for each nursery followed by the same letter are not significantly different at alpha = 0.05.

Treatment	# White root tips	Root volume cm³ (in³)	Root length cm (in)	# Root tips	# Root forks	Root weight ratio
Nursery A (slash pine)						
Hand-lifted (control)	78.1 a	2.30 (0.140) a	293 (115) a	814 a	1,488 a	0.16 a
Mathis® two-row—normal speed	51.0 b	1.68 (0.103) b	215 (85) bc	746 ab	894 b	0.14 a
Mathis® two-row—fast speed	53.8 b	1.66 (0.101) b	202 (80) bc	582 b	886 b	0.15 a
Love® full-bed—normal speed	47.5 b	1.96 (0.120) ab	240 (94) bc	593 b	945 b	0.15 a
Love® full-bed—fast speed	88.0 a	2.04 (0.124) ab	254 (100) ab	585 b	1,101 b	0.15 a
Nursery B (loblolly pine)						
Hand-lifted (control)	63.5 a	3.81 (0.232) a	353 (139) a	742 a	1,916 a	0.24 a
Love® full-bed—normal speed	61.1 a	2.20 (0.134) c	206 (81) c	466 c	907 c	0.23 a
Love® full-bed—fast speed	74.1 a	2.75 (0.165) b	255 (100) b	580 b	1,204 b	0.23 a
Nursery C (loblolly pine)						
Hand-lifted (control)	34.1 b	3.72 (0.227) a	441 (174) a	847 a	2,402 a	0.23 a
Love® full-bed—normal speed	45.1 a	3.79 (0.231) a	383 (151) a	727 a	1,845 a	0.25 a
Love® full-bed—fast speed	26.5 c	3.78 (0.231) a	431 (170) a	776 a	2,058 a	0.24 a

treatments had greater RGP than either speed used on the Mathis® two-row lifter and the slow speed on the Love® full-bed lifer (table 3). Seedling root volume, root length, and number of root forks were significantly less for the Mathis® two-row lifter compared with the hand-lifted controls. No difference existed between the root volume from the hand-lifted controls and the Love® full-bed lifter (table 3).

Nursery B

Lifter speed had no effect on number of white root tips, RWR (table 3), RCD, height, root biomass, or shoot biomass (data not shown). In contrast, root volume, root length, number of root tips, and number of root forks were significantly greater on seedlings lifted at the fast speed compared with those lifted at the slower (normal) speed (table 3). Hand lifting seedlings at this nursery resulted in greater root volumes, root lengths, number of roots tips and forks when compared with either speeds of the full-bed lifter (table 3).

Nursery C

The lifting speed had no effect on root volume, root length, number of root tips, number of root forks, or RWR (table 3). In addition, RCD, height, root biomass, and shoot biomass were similar between lifting speeds and the hand-lifted controls (data not shown]. In contrast, the full-bed lifter operated at the normal speed had more white root tips than either the full-bed lifter operated at fast speed or the hand-lifted controls (table 3).

Discussion

Personnel operating forest-seedling nurseries routinely calibrate their seedling lifter before the lifting and packing season. Comparing the root mass of seedlings lifted with a shovel from an adjacent area with those from the seedling lifter is the most common method for evaluating seedling lifter efficiency (Langdon 1954). The goal is to have a fibrous root system equal or better than hand lifted (Darby 1962). This method is very subjective, and detecting root loss, especially of fine and mycorrhizal roots, may be difficult. In our study, no differences were observed among treatments for root biomass or RWR at any nursery; however, treatment differences were observed for RGP and other root morphology characteristics. South and Stumpff (1990) showed that even a small loss of fine roots, not reflected in root weight, can result in up to a 50-percent reduction in RGP.

One of the more interesting results was that seedlings lifted by the Love® full-bed lifter had larger RCD compared with those lifted by the partial-bed Mathis® lifter. This larger RCD is likely because of the difference in seedling size within the seedling drills and the seedlings sampled by each lifter. In a typical nursery bed, the two outside drills (drills 1 and 8) have larger RCD than the inside drills (drills 2 to 7). Thus, the Love® lifter sampled the entire nursery bed, whereas the Mathis® two-row lifter lifted seedlings from the interior rows, using drills 3 and 6, which tend to be smaller (figure 4). Although the difference was statistically significant, a 6-percent difference would not be biologically significant.

Although no differences were observed in RWR at any of the nurseries with respect to lifting speed, a difference in the magnitude of RWR was observed among nurseries. A RWR of more than 27 percent is equivalent to a shoot-to-root ratio of 2.5:1.0, an optimum ratio for outplanting survival (USDA Forest Service 1989). While none of the lifting speeds or lifters examined in these trials resulted in the optimum RWR at the time of lifting, a number of factors may have influenced the RWR. For example, the time of lifting, the seeding density, the time of root pruning (lateral and undercutting), the irrigation regime, and the time since fertilization can all affect the RWR. At Nursery A, when the seedlings were lifted in December, the average RWR was 15 percent whereas at the other two nurseries, where seedlings were lifted in February, the average RWR was 24 percent. Sung and others (1997) showed that typical southern pine RWRs from September to February can range from 11 to 28 percent and can increase up to 25 percent per month. Because of the various cultural practices conducted within a nursery, it is difficult for nurseries that lift seedlings in October or November to obtain RWRs near 27 percent. Because RWR is correlated with survival after outplanting (South 1998), the loss of roots or damage to the root system in nurseries with low RWR (low root biomass) may have the potential for poor outplanting performance compared with seedlings with a greater RWR (greater root biomass).

Most nurseries in the Southern United States no longer use a two-row lifter because of the amount of fine roots remaining in the soil after lifting. In addition, four passes must be made over the same bed to lift all seedlings, resulting in even more root damage. In this study, the use of the Mathis® two-row lifter at both speeds resulted in lower RGP, root volume, root length, and root forks when compared with hand-lifted controls. Similar reductions in root morphology as well as decreased outplanting survival, 1-year volume, height, and diameter were reported by Reynolds and others (2002) when loblolly pine seedlings lifted with a Mathis® two-row lifter were compared with hand-lifted seedlings. In another study, second-year survival, height increment, and volume index were significantly less with a Mathis® two-row lifter compared with hand-lifted controls (Greene and Danley 2001). South and Cary (2001) reported outplanting survival of loblolly pine from a two-row lifter was reduced by 40 percent compared with the hand-lifted controls.

In this study, the normal speed used for each lifter was not always the most efficient as measured by RGP and/or root morphology characteristics. At Nursery A, the full-bed lifter at the faster speed had greater RGP than seedlings lifted at their normal, operational speed. At Nursery B, all root morphology characteristics were greater on seedlings lifted at the faster speed than seedlings lifted at the normal, operational speed. Based on these seedling root characteristics, better seedling quality may have been achieved at Nurseries A and B if the lifter had been calibrated at a faster tractor speed before the onset of the lifting season. Care should be taken to ensure the belt speed and tractor speed result in the greatest amount of roots per seedling to ensure seedling survival after outplanting. The best RGP and root morphology data would be expected when the belt speed properly matches the tractor speed. This observation was made at Nursery C, where the RGP at the normal (calibrated) speed was greater than at the faster speed with no other detectable difference in root morphology measurements.

Of the lifters examined in these trials, Nursery C was the only nursery to use the lifter blade during operation. This particular nursery has a finer textured soil than the other two nurseries (table 1) and was the only nursery where the hand-lifted seedlings resulted in a lower RGP than the machine-lifted seedlings. Lower RGP on hand-lifted seedlings is counterintuitive and one explanation may be the lateral and undercutting process at this nursery. When we hand-lifted the control seedlings, only vertical shovel cuts were made, and, although the seedling beds had been undercut several months earlier, the roots in the fine-textured soil continued to grow and were difficult to remove from the soil with a shovel without root damage. In addition, when the fast speed was used with the lifter blade, the seedling belts did not pick up individual seedling but rather, large clumps of seedlings were lifted at a time. Running the tractor at the faster speed caused the seedlings to jam the seedling belt as described by Darby (1962). Making a corresponding adjustment to the seedling belts would have compensated for the faster tractor speed. The lifter at this nursery was calibrated for the normal speed using the lifter blade, which may explain the lack of differences in the root morphology characteristics.

Conclusions

Calibrating the belt speed on a seedling lifter so that seedlings are individually removed from the nursery bed without injury is critical. When using ocular comparisons to evaluate seedling lifter efficiency, nursery staff need to give careful attention to the presence or absence of fine root tips, mycorrhizae, damage to the root collar region, and any possible breaks in root cortex on lateral roots. Even minor root damage can reduce RGP and negatively affect outplanting performance. The use of the lifter blade on the full-bed lifter may help to increase seedling quality on other soil types by reducing the loss of fine roots but tractor speed must be matched to the belt speed to minimize root damage.

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A Water Activity-Regulated Dryer: How To Dry Seeds or Pollen With Water and No Heat

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Abstract

The hydrous status of seeds and pollen can now be characterized more precisely than moisture content through the measurement of water activity (a_w). This new technique, now available to managers of genetic resources banks, offers the advantage of being fast, easy to use and, above all, nondestructive. This article describes components and use of an a_w-regulated seed/pollen dryer developed by the National Research Institute of Science and Technology for Environment and Agriculture (Irstea, France). The simple design of this highly versatile heat-free dryer allows homogenous drying of seed and pollen lots at an a_w level that is safely compatible with reliable medium- and long-term conservation. The *ex situ* conservation of biodiversity is a major issue in the context of climate change. Hence, the dryer proves to be a high-performance tool in readjusting the hydrous status of lots likely to change during conservation.

Introduction

The measurement of water activity (a_w), often associated with the measurement of equilibrium relative humidity (ERH), is a technique developed and widely used by the agrifood industry (Barbosa-Canovas and others 2007). This technique allows for characterizing interactions between water and other component molecules and predicting their potential for conservation. One property of hygrophilous materials is the ability to capture or yield water depending on the ERH of surrounding environmental conditions. At equilibrium, the a_w qualifies the water status in the material, while the ERH qualifies the surrounding environment conditions. In more technical terms, the a_w is assessed by measuring the ERH on immediate contact with the sample; hence, the two measurements are often related. The a_w is the ratio between the water vapor pressure of a sample and the pressure of pure water, and ranges from 0 to 1. This ratio ranges from 0 to 100 percent when expressed in terms of ERH. This measurement technique is fast, reliable, reproducible, and nondestructive. In addition, operators only need basic training to measure a_w.

In general, the prestorage hydrous status of organic materials, referred to as intermediate humidity, is an influencing factor in their conservation and longevity. To date, the hydrous status of forest reproductive materials (seed and pollen) has been generally assessed by measuring mass moisture content. But this measurement technique is time consuming, destructive, and only quantitative. In addition, the chemical availability of water and its potential adverse effects on the conservation of stored lots cannot be qualified with this technique. Conversely, the measurement of a_w provides an accurate assessment of the deterioration risk related to water status (Baldet and others 2009).

Since 2004, Irstea (The National Research Institute of Science and Technology for Environment and Agriculture, France, formerly known as Cemagref) has demonstrated the usefulness of a_w in the forest genetic resources field through the application of this technique to the control of seed or pollen hydrous status (Baldet 2006). The degradation agents of seeds and pollen, whether biotic (bacteria and mold) or abiotic (oxidation and enzymatic reactions), are not dependent on the amount of mass moisture contained in a given compound, but on the chemical availability of water qualified by the measurement of a_w (figure 1). For instance, humans have known for thousands

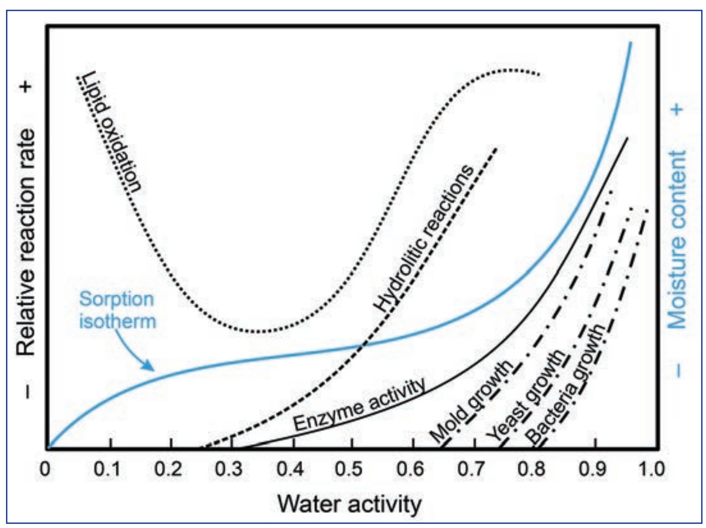

Figure 1. Schematic diagram of the main degradation agents of organic materials associated with the chemical availability of water. (Adapted from Labuza and others, 1972)

of years how to prolong the preservation of foods by adding "water binders" such as salt or sugar to reduce the fraction of chemically available water.

Through collaborative work, Irstea and the Direction de la recherche forestière (DRF) of the Ministry of Natural Resources (MRN) of Quebec determined the universal a_w value of 0.35 for the safe storage of orthodox seeds and pollen (that can be safely dried) by describing the hydrous behavior of hundreds of seed and pollen samples (Colas and others 2010). The a_w 0.35 value is a noteworthy value at which the main degradation factors are the least active (figure 1). Therefore, this value helps to prevent the development of any biotic agents and to hold chemical reactions at their lowest levels. Forest reproductive materials provide a wide range of applications to control "active" hydrous status using a drying technique or by using a "passive" hydrous status through the measurement of a_w. Controlling hydrous status allows for the conservation of seed or pollen lots for operational, research, or genotype conservation purposes.

Forest tree seed crops can be irregular. As a consequence, reforestation, genetic improvement, and genetic resources seed bank programs must operate within available perennial harvests. Resorting to conservation procedures is therefore unavoidable and is critical to the success of a viable seed bank program (Probert 2003). Orthodox seed and pollen conservation processes involve multiple stages (initial open-air drying, post-maturation, extraction, and final drying) during which the hydrous status of seeds and pollen should be closely monitored. Seed and pollen are conventionally dried using heat. This drying process must be properly controlled to avoid excessive drying likely to lower the short- and long-term quality of lots. Dried materials are not preserved in a stable and definitive state. The conservation of dried materials is a rather slow and imperceptible, yet dynamic, process during which the permeability of storage containers, the environmental conditions of conservation, or the intrinsic conditions of the lots can change the initial hydrous status into values involving a potential risk of degradation (Colas and others 2012). Therefore, the medium- and long-term management of seed and pollen conservation requires controlling and adjusting the hydrous status of stored lots on a regular basis.

Irstea developed a seed/pollen dryer that is regulated by the a_w parameter that allows perfect coherence between the management and final control of hydrous status (Baldet 2006). This simple, reliable, fast, and reproducible integrated drying and control method can also be a component of an overall quality assurance project. Following the transfer of this technology as part of a scientific and technical collaborative agreement signed between Irstea and the MRN, the DRF built and implemented several seed/pollen dryers using the information and plans provided by Irstea. These dryers have become invaluable tools in the routine daily management of seed and pollen lots in Quebec (Colas and Bettez 2012).

The purpose of this article is to present the general working principle and operating mode of the seed/pollen dryer and the functions of its different components, so that operational units that process seeds or pollen can build the dryer locally and can easily operate and maintain it.

Two-Pressure Principle

The a_w-regulated seed/pollen dryer works under the fundamental two-pressure principle developed originally by the National Institute of Standards and Technology (Hasegawa and Little 1977). This method is used as a standard relative humidity generator by saturating air with humidity under given controlled pressure and temperature and, as a second step, expanding the saturated air at the required operating pressure (figure 2). The resulting ERH (or a_w) equals the ratio of the two monitored total pressures (Equation 1). Post-expansion pressure ("*p*") is not necessarily the value of atmospheric pressure, but an additional parameter used to adjust the final ERH.

$$\text{ERH}\ (a_w) = \frac{p}{p_1}$$

p = Post-expansion humidified air pressure.

p_1 = Total pressure during the stage when air is saturated with humidity

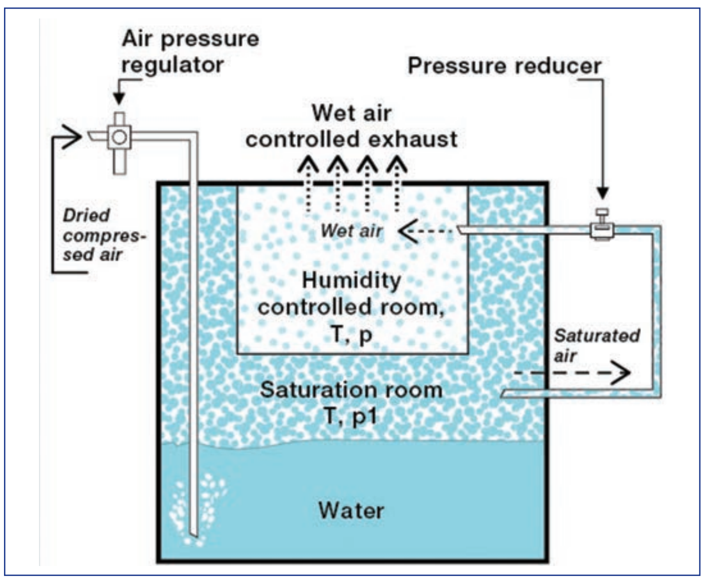

Figure 2. Schematic diagram of a two-pressure reactor. T = temperature, p1 = saturation pressure, and p = post-expansion air pressure. (Adapted from HUMOR 20 High-precision Humidity Calibrator data sheet, E + E Elektronik Ges.m.b.H, Langwiesen 7, A-4209, Engerwitzdorf, Austria)

Based on this principle, Irstea designed an a_w-regulated dryer. The diagram in figure 3 shows a simple version of the device with a set, manually preadjusted saturation pressure. An “automated” version of the dryer adjusts the saturation pressure automatically in regard to the effective ERH measured on an ongoing basis inside the drying cabinet (Baldet 2006). Only the simple version of the dryer operated at a set saturation pressure is discussed in this article. The authors wish for this technique to generate accurate results at a satisfactory level, while remaining technologically simple and cost-effective to simplify the construction and routine management of this device. Figure 4 shows the device built at the DRF using the information provided by Irstea.

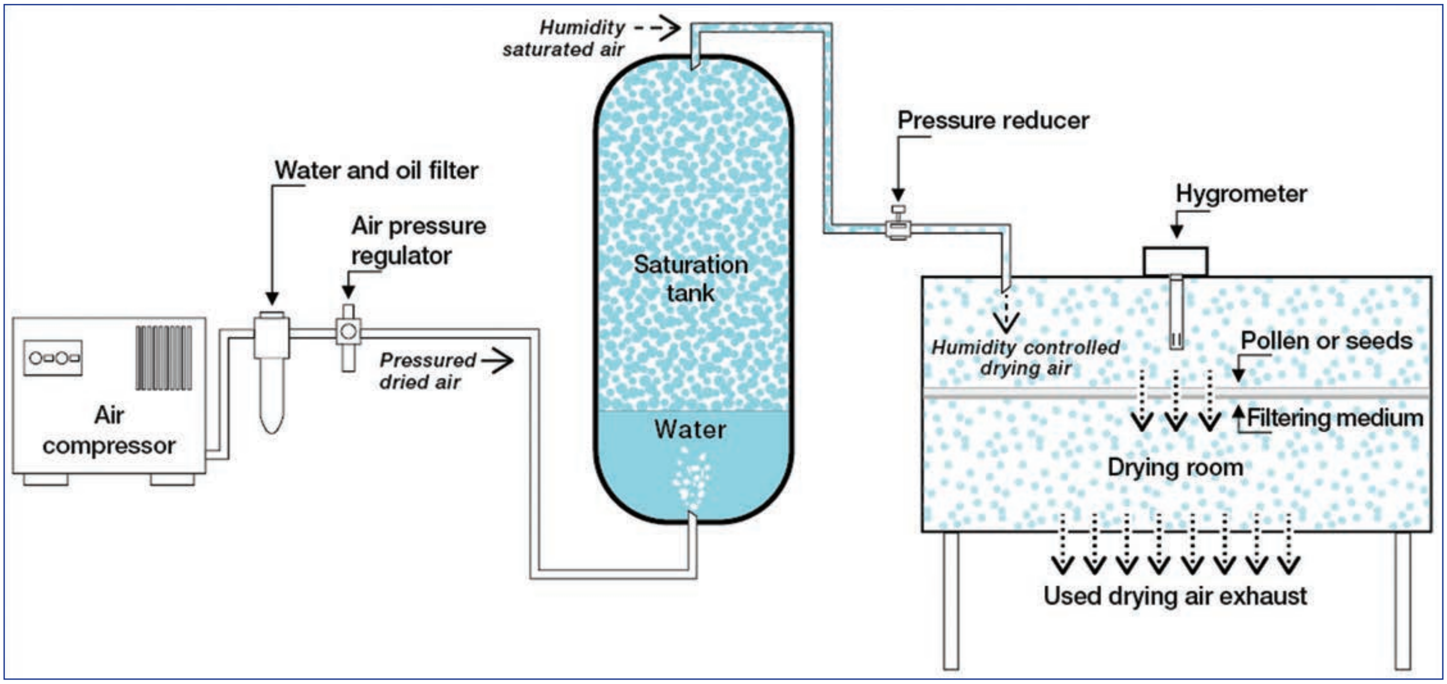

Figure 3. Schematic diagram of the Irstea dryer. The saturation pressure is set and manually preadjusted. (Adapted from Baldet, 2006)

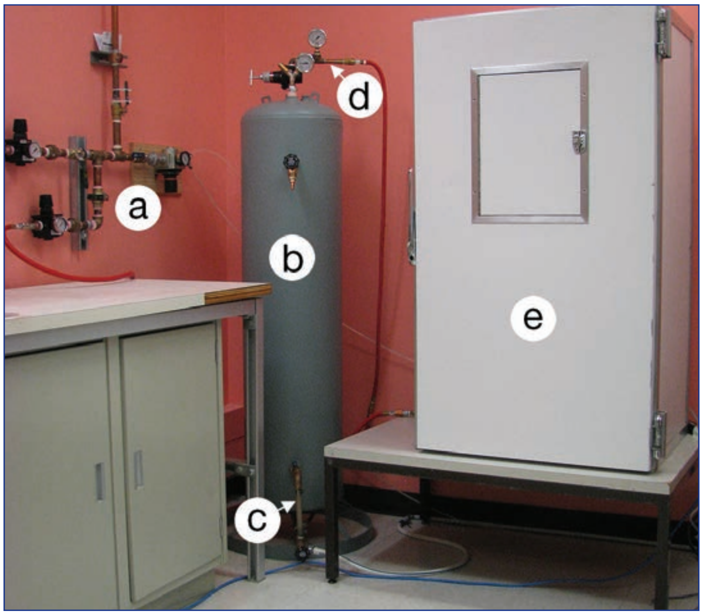

Figure 4. A general view of the device installed at the laboratory of the Direction de la recherche forestière. (a) Compressed air supply (in this case, supplied by the building system); (b) saturation tank (air humidification); (c) control tube showing the level of water in the saturation tank; (d) outlet for driving air at a predetermined level of a_w into the drying cabinet; (e) drying cabinet. (Photo by Fabienne Colas, 2012)

Design and Operation Details

Different technical stages were involved in the design of the seed/pollen dryer regulated by a_w (or at a controlled ERH). A detailed description of each stage follows. The characteristics of the main components needed to build the dryer can be provided on request. (Please contact Patrick Baldet.)

Saturation Tank

The tank used for air saturation ideally has an inner wall protected with a coating (e.g., enamel, paint) to prevent corrosion because of the presence of water and the constant renewal of dissolved oxygen. Water used in the tank must be at room temperature before the dryer is operated. It is therefore recommended to fill the tank at least 1 day before operations to stabilize the water temperature to that of the room where the dryer is installed. The water level in the tank can be easily monitored through the installed control tube (figure 4). The amount of water required depends on the expected time of use of the dryer. In the conditions discussed in this article (tank of about 32 gal or 120 L), the dryer was operated at an a_w of about 0.35 during one uninterrupted month with an initial water volume of about 5 gal (about 20 L).

The standard operation of the unit is also likely to increase the amount of water in the saturation tank if the ERH of the air expelled into the drying cabinet is below the ERH of the air pumped by the compressor. In our case, the output ERH was 35 percent (resulting a_w of 0.35) for an average ambient ERH of 50 percent.

Saturation Pressure Adjustments

Air saturation pressure inside the tank must be optimized for each installation. The pressure will vary slightly depending on tank characteristics, the accuracy of saturation pressure measuring devices, and compressed air supply.

To optimize air saturation pressure in the specific conditions of each laboratory, it is recommended to develop a curve relating the a_w generated by the expansion of pressurized air to the saturation pressure in the tank (figure 5). The first expansion pressure at the tank outlet is set at 5 psi (0.34 bar), which represents a 13 gal/min (or 50 L/min) air flow for 0.25 in (6 mm) piping not exceeding 6 ft (2 m) in length between the air expansion point and the drying cabinet. This average flow is recommended for a 5 ft^2 drying surface (about 0.5 m^2), representing an average air-flow rate of 4 in (10 cm) per minute across the section of the drying cabinet. The required tank saturation pressure can be set and adjusted according to the desired a_w. A pressure of 18 to 34 psi (1.3 to 2.3 bar) is required to obtain a 0.4 to 0.3 a_w (figure 5).

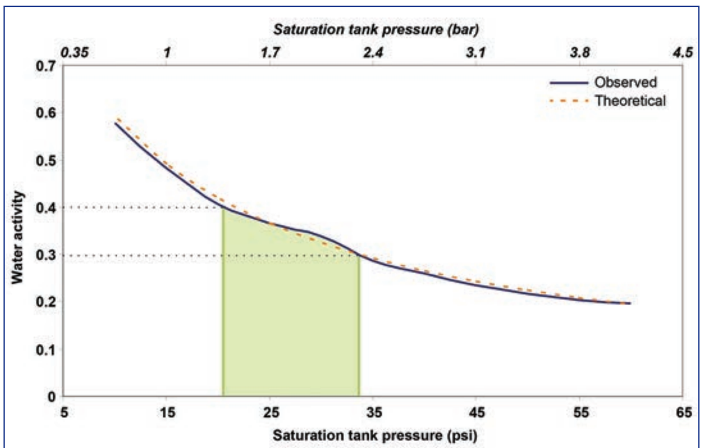

Figure 5. Water activity (a_w) of drying air, measured and theoretical, at the outlet of the dryer with relation to the saturation pressure in the tank (in psi or bar). The expansion pressure of air at the dryer outlet is set at 5.00 psi (0.34 bar). In green is the target range of pressures in the saturation tank necessary to obtain a level of a_w between 0.3 and 0.4 required to dry seeds or pollen. The theoretical a_w values were calculated using Equation 1. (Barbosa and others 2007)

The dryer described in this article was designed for safe, heat-free seed or pollen drying to limit damage sustained by materials that are still immature or that have only partly acquired physiological resistance to drying (Hay and Smith 2003). The wide diversity of forest reproductive materials increases the likelihood of having to manage in a single procedure lots containing seeds and pollen with different levels of tolerance to drying.

As shown in figure 5, the curves of both observed and theoretical values practically merge. In fact, they are identical if the mean accuracy of a_w measurement devices (±0.02) and type 1 medium accuracy-class manometers (±1 percent) are integrated. This illustration shows that the application of theoretical saturation pressure values is highly reliable. Measuring a_w systematically at the outlet of the drying cabinet is not necessary because only the accurate adjustment and measurement of the saturation pressure is sufficient. Therefore, the dryer can be operated without any complex electronic measuring instruments.

The Drying Cabinet

The material to use for the drying cabinet structure must not be hydrophilic, and it should be smooth and washable. Wood should be avoided. A good compromise is to use stiff plastic such as KömaCel® (Kömmerling Kunststoffe, Pirmasens, Germany), which interacts very little with the a_w of drying air and is easy to cut and assemble. Any other plastic material sufficiently stiff can be used. A laboratory oven can even be converted into a dryer after removing the electrical equipment and making minor modifications for the intake and outlet of drying air. The dimensions of the drying cabinet depend on the future use of the equipment (number of lots, volume of lot units, purity requirements, etc.).

Drying air will enter the cabinet at the base and will exit at the top, following the natural upward movement of moist air. The drying air is introduced inside the cabinet through pipes punctured at intervals of 2 to 4 in (5 to 10 cm), so that the drying airflow distributes evenly (figure 6). This method is well suited for drying small lots with high purity guaranteed by individual conditioning, allowing for dried materials to be in indirect contact with the main flow of drying air. To dry large amounts of material covering most of the useful surface of the dryer with no or little purity constraints, the direction of drying air supply can be more efficient if flowing from top to bottom (figure 3). Therefore, the drying air will directly flow through the layer of materials laid out to dry. This downward air supply mode is particularly well suited to light products like pollen or winged seeds that will be flattened against the drying surface.

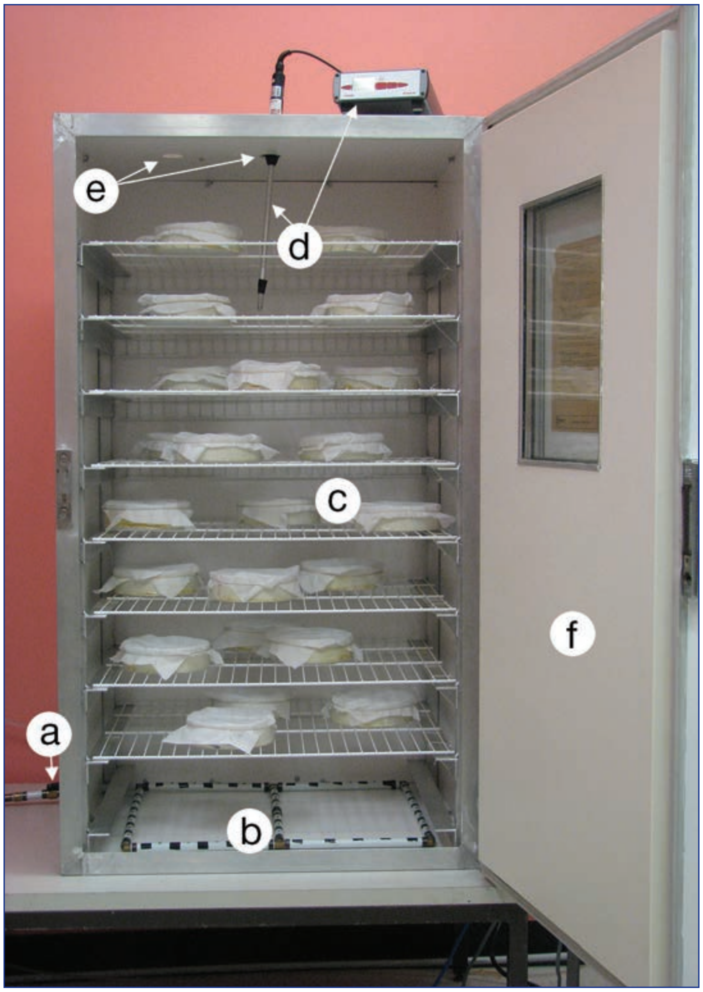

Figure 6. Drying cabinet built at the Direction de la recherche forestière. (a) Drying air intake at controlled humidity levels, (b) air intake suitable for the final expansion of drying air, (c) containers used for drying pollen lots, (d) hygrometry probe in the drying cabinet, (e) outlets to evacuate drying air after use, (f) drying cabinet door. (Photo by Fabienne Colas, 2012)

In the drying cabinet, the position of the a_w measurement probe in relation to air intake is important. If the probe is placed relatively close to the air intake (lower part of the cabinet), it will measure the a_w of the drying air and will show the proper operation of the technical process. If the probe is placed opposite the air intake (position shown on figure 6, on the upper part of the cabinet), however, the probe will measure the a_w after exchanging with the materials during the drying process and will then represent the drying interactions of materials treated with higher values at the onset of the process, if a large quantity of moist materials is inside the cabinet. The position close to the air outlet is preferable because it enables to more accurately monitor the drying of materials.

Operations

After water in the tank is at room temperature, open the compressed air intake and adjust the tank saturation pressure at the value determined during preliminary adjustments while keeping the outlet closed. The expansion pressure at the outlet of the tank will be adjusted after the required pressure is achieved in the tank. When the required pressure is achieved, open the air intake in the drying cabinet and verify the ERH value. If needed, adjust the saturation pressure to achieve the desired ERH value and, consequently, the resulting a_w required for the products to dry. After adjusted, the ERH remains very stable. Because most hygrometers provide measurements at a precision of ±2 units of ERH, this measurement uncertainty should be considered in any new adjustment of the saturation pressure. Overall, the saturation pressure is always easier to measure than ERH. Using a hygrometer is not absolutely necessary for dryer operations; hygrometers are only a means to control actual saturation pressure.

Drying

To optimize drying efficiency, samples, whether seeds or pollen, should be laid out in relatively thin layers in a container permeable to air. We use and recommend a polyamide monofilament textile mesh produced by the SAATI Company (Appiano Gentile, Italy).

When samples have different initial a_w values, the driest samples should be placed in the cabinet next to the drying air intake, and the dampest samples should be placed next to the air outlet to avoid rehydrating already stabilized samples.

The time needed to dry samples depends on their initial hydrous status. To ensure a final stable sample value, it is preferable to leave samples slightly longer in the dryer. After the a_w value imposed by ERH-controlled air is achieved, the samples will maintain this value even if they remain for a relatively long time in the dryer (figure 7).

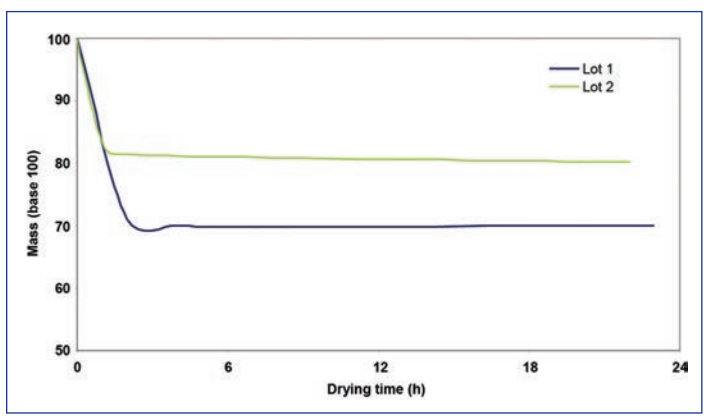

Figure 7. Stabilization of the mass of two Japanese larch pollen samples at a drying a_w level of 0.35. In such cases, the pollen is dry after about 2 to 4 hours in the dryer. Their mass, and therefore their a_w-qualified hydrous status, remains at a constant level for up to 24 hours in the dryer. (Philippe and others 2006)

Lot Storage

When removing the samples from the dryer, the lots must be quickly placed in vapor-proof storage containers to minimize water uptake. To make it easier to handle samples and to limit water uptake, the room where the dryer is set up must ideally have an ERH value close to the value applied to samples in the dryer.

Operational Advantages

- Based on its underlying principle, the drying process discussed in this article aims at achieving equilibrium between the ERH of drying air and the a_w of treated materials. With this technique, seeds or pollen can remain inside the drying cabinet without any risk of drying beyond the required value, because the equilibrium value remains constant after it is reached. This is a significant advantage when treating samples during an intensive period of activity often associated with climatic and phenological constraints. In fact, this technique allows easier management of technical resources assigned to a drying operation, particularly for pollen. Therefore, there is no need to mobilize several people to treat samples quickly. Leaving a sample slightly longer in a dryer will not impact its quality, because this technique defines a lower technical threshold of a_w, unlike that observed when samples are dried using the conventional method of heat application.
- The seed/pollen dryer stabilizes samples at a predetermined a_w necessary for conservation. As the samples are dried without the application of heat, achieving equilibrium is safer for the biological material and more progressive than conventional drying. Storage at low temperatures can be engaged quickly without a cooling stage highly conducive to untimely water uptake.
- Climate change issues are generating more interest in long-term conservation of seeds and pollen. The hydrous status of preserved lots can change because of container permeability, aging, etc., during conservation. Owing to the nondestructive advantage of measuring a_w, the quality of lots preserved in banks can be controlled on a regular basis without reducing initial stored amounts. Any significant increase in a_w will lead to the degradation of lot quality (figure 1). Therefore, lots with a raised a_w can be restabilized at the optimal a_w of conservation in the dryer, to be then placed back into the bank. This technique can help ensure the quality of seed and pollen lots over the long term thus enhancing the potential of regenerating lots for future reintroduction.
- This drying process is based on an accurate, yet basic method. Only a quality pressure regulator is needed to ensure a reliable and continuous relative humidity of the drying air. Because it is so technically simple to use, the dryer is particularly intended for point-to-point or seasonal applications during which operators' control, calibration, and training costs must be as minimal as possible.

Operational Limitations

- The production of compressed air is a low energy-efficiency operation because air compression is an exothermic process requiring a large amount of energy. Therefore, the drying process involving the production of air at a level of ERH controlled by the two-pressure technique should be restricted to small drying units and seasonal applications. In addition, as shown in figure 5, obtaining the lowest ERH values requires increasingly higher pressures. Because this process is not a linear function, but a kind of asymptotic function in which the production of very dry air at 1 percent ERH would theoretically require a saturation pressure of 1,430 psi, it is appropriate to restrict the use of this method to relative humidity values above 20 percent (a_w values above 0.2).
- Dryer operations are based on the availability of filtered, de-oiled compressed air. For instance, operations may require dedicated air compression equipment if the "drying room" is not connected to a compressed air production and distribution system.
- Under national legislation, very strict regulations dictate the use of pressure equipment. In particular, regulations pertaining to the exclusive use of certified and tested materials are also subject to periodic official controls. These regulatory constraints must be considered at the early stages of any a_w pollen and seed dryer project and particularly when choosing the equipment to implement.

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Resolving Western White Pine Seed Germination Differences Between Lab Testing Protocols and Operational Greenhouse Protocols at Webster Nursery

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Abstract

The efficient production of western white pine seedlings (*Pinus monticola* Douglas ex D. Don) has historically been a challenge for growers in the Northwestern United States. Great progress has been made in unlocking the dormancy of this species, resulting in better, more uniform seedling crops, but room for improvement remains. This article addresses the discrepancy between seed stratification protocols for lab germination tests, on which sowing calculations are based, and stratification protocols for operational sowing. The goal was to develop a repeatable lab stratification approach to mirror as closely as possible the results of improved operational stratification methods. Results showed that alternative lab stratification approaches outperformed standard lab protocols, thereby providing a better measure of operational greenhouse performance. Comparisons between this study, which was performed without bleach as a surface sterilant, and an earlier trial of the same seedlots using a bleach rinse, suggest that a surface treatment can elevate germination capacity in western white pine.

Introduction

The deep seed dormancy of western white pine (*Pinus monticola* Douglas ex D. Don; WWP) has resulted in low nursery performance expectations relative to other conifers in the northwestern United States for many years. Extensive research into stratification techniques by the British Columbia Ministry of Forests Tree Seed Centre (BCTSC); now the BC Ministry of Forests, Lands, and Natural Resource Operations) and operational greenhouses in recent years has brought the quality and consistency of nursery crops to a level comparable with other species (Kolotelo 2013). These advances are significant, because the high cost of WWP seed bred for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch), along with the large seed size of this species and its historically inconsistent germination performance, combine for very high per-seedling costs.

Despite recent advances, some difficulties in overcoming dormancy in WWP still remain. The WWP germination testing protocols used by seed labs in northwest North America accredited by the International Seed Testing Association (ISTA) have not been modified to reflect recent research and continue to report chronically low germination capacities. On the other hand, container nurseries in northwest North America have modified operational WWP germination protocols over the years in response to ongoing research and now achieve high germination capacities and vigorous germination rates. The problem lies in where these two processes come together. Seed sowing calculations are based on lab germination capacities rather than operational greenhouse germination capacities. As a result, WWP sowing rates (based on lower germination capacities) are often higher than they need to be to achieve the desired number of seedlings, resulting in the waste of expensive seed. Seeds that would not normally be released from dormancy under current lab stratification protocols germinate successfully under operational protocols, which results in higher greenhouse thinning costs as the excess seeds germinate at higher rates than predicted by lab data (figure 1).

Figure 1. Operational greenhouse germination of Seedlot 643, showing multiple germinants per cell. (Photo by Jeff deGraan, 2013)

The discrepancy in germination protocols has a genetic component as well. Seeds subjected to stratification protocols, which result in uniform dormancy release followed by homogeneous germination, are given the opportunity to initiate growth concurrently. Differences in seed dormancy levels as well as in germination speed result in size differences among multiple-sown germinants, and greenhouse thinning to leave the largest germinants will likely favor parents that produce less dormant, faster germinating seed (Edwards and El-Kassaby 1996). For WWP, the favored characteristics at thinning in the nursery may not be aligned with those that promote blister rust resistance in the woods, so some families displaying resistant attributes could be lost.

Project Objectives

Our goal with this project was to develop a new lab stratification protocol to better mirror current operational stratification practices at the Washington Department of Natural Resources Webster Nursery Greenhouse (WA DNR Webster Nursery) and thus optimize seed use and reduce associated costs. A successful lab protocol will meet the following criteria:

- Identify the variables that can impact germination.
- Reflect operational protocols so that factors such as moisture content, stratification, and germination assessment are comparable.
- Consistently reflect germination performance of operational stratification protocols with an acceptable degree of accuracy.

Similar efforts to reconcile lab and nursery results have been made by the BCTSC and others (Danielson 1985, Kolotelo 2001, Kolotelo and others 2001). To place official lab testing protocols in perspective, seed testing is not intended to reproduce the conditions under which seed may be sown, rather, it seeks to produce conditions and procedures so that results of different labs can be compared (Edwards and Wang 1995). One of our goals is to determine if a consistently repeatable lab protocol can return results in line with operational nursery results.

If successful, this new lab method should better reflect the optimum germination capacity of WWP seed at WA DNR Webster Nursery, taking into consideration processes specific to that facility. Development of the optimum protocol should result in greater seed-use efficiency, reduced greenhouse labor costs, and potential expression of a fuller array of blister rust-resistant parents.

Variables Influencing Comparisons Between Lab and Operational Greenhouse Germination Practices

For this project, variations exist not only among the treatments, but also between our proposed protocol and the operational practice it is designed to try to reproduce. Therefore, results will likely be dependent on how practices at individual nurseries are conducted. The following paragraphs describe some of the potential sources of variation that influence seed germination protocols.

1. Variations in seedlots

   Seed dormancy varies among seedlots as a result of differences among trees and stands, crop years, and in response to cone collection timing (Kolotelo 1997, 2002a; Wang and D'Eon 2003). Most of these variables cannot be controlled.

2. Variations in imbibition between operational greenhouse practices and lab trial methods

   In operational stratification, seeds are often placed into 1.0 to 5.0 gal (3.8 to 18.9 L) mesh bags, keeping them in contact with their neighbors on all sides and providing fairly consistent and resilient moisture levels during stratification (Kolotelo 2001). Were this same approach downsized to accommodate an operational lab trial, one could expect smaller mesh bags to result in stratification moisture levels that were more variable and less resilient. In the lab, germination trays are used for standard germination testing, and seeds are isolated from the outside environment but no contact is made among them. As a consequence, moisture levels could vary significantly from those of the operational approach. Kolotelo (2001) found that lab germination tray samples at the BCTSC were maintained in stratification with an average moisture content 3.4 percent higher than operational greenhouse seedlots.

   Other variables related to moisture content that should be taken into account are the effect of water temperature on imbibition (Kolotelo and others 2001, Feurtado and others 2003) and the precision of the moisture meters employed in making comparisons between methods.

3. Variations in germination environments between operational greenhouse practices and lab methods

   Lab germination is conducted in closed containers in a clean, controlled environment, while greenhouse germination takes place in a less clean, more variable environment. Temperature ranges can be greater in a greenhouse setting as well (Kolotelo and others 2001) and daily photoperiod is manipulated to promote growth. Operational greenhouse germination at Webster Nursery takes place in conditions of 16 to 18 hours of daylight and 6 to 8 hours of darkness, with a mean temperature of 70 °F (21 °C) for both day and night. The temperature in this setting can occasionally reach 85 °F (29 °C) depending on outside environmental conditions. Operational lab germination for the purposes of this study occurs in alternating cycles of 86 °F (30 °C) in daylight for 8 hours and 68 °F (20 °C) in darkness for 16 hours. One could expect these environmental variations to result in differential germination rate and capacity.

4. Variations in how germination is defined

   One difficulty cited by Kolotelo and others (2001) in making a comparison of lab germination and operational germination is the different measures of success between the two. By lab standard, a seed is germinated when its radicle length has extended to four times the seed length. In the greenhouse, a seed is characterized as germinated when its cotyledons are unfolded and have begun photosynthesis, at which time the radicle is approximately 10 times the seed length. It is conceivable that a seed defined as germinated in a lab setting may never reach the greenhouse threshold.

   The trial described in this article is a step in the process toward evaluating the impact of some of these variables, with an eventual goal of identifying the best pairing of an optimal operational protocol with a lab test protocol.

Materials and Methods

Eight WWP seedlots were chosen for the trial. Five of these seedlots were from the Inland Empire Tree Improvement Cooperative's R.T. Bingham seed orchard (Moscow, ID) and the other three were from the U.S. Department of Agriculture, Forest Service Coyote Seed Orchard (Vancouver, WA) and Dennie Ahl Seed Orchard (Shelton, WA). The lab germination trials were developed in collaboration with WA DNR Webster Nursery and were conducted at the WA DNR Seed Center (both in Olympia, WA).

Three stratification treatments were included in the trial:

1. Association of Official Seed Analysts (AOSA) Standard—A 24-hour soak with 90-day cold stratification in germination trays.
2. Webster Operational-based—A 14-day running-water soak in mesh bags followed by 30-day high moisture content, then 90-day low moisture content, cold stratification regime.
3. Low Moisture Operational-based—A 14-day running-water soak in mesh bags followed by a low moisture content regime.

Treatment 1—AOSA Standard

This protocol was based on AOSA "Rules for Testing Seeds" (AOSA 2007), which have been validated by the ISTA. Representative 400-seed samples for each of the 8 seedlots were collected and soaked for 24 hours in a capped plastic vial filled with tap water at room temperature 64 to 72 °F (18 to 22 °C). No seedcoat sanitation process, such as bleach or hydrogen peroxide, is described in the AOSA guidelines. The excess water was drained from the vials to bring the samples to an initial moisture content of 45 percent as measured by a Steinlite model 400G moisture meter. Seeds from each seedlot were then split into eight 50-seed samples and spread out evenly onto Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, labeled by treatment, and placed into a cooler at 35 °F (1 to 2 °C) for 90 days' stratification before placement in a germination chamber.

Treatment 2—Webster Operational-Based

The Webster Operational-based lab protocol follows WA DNR Webster Nursery's operational naked stratification for WWP, which was developed from the method described in the British Columbia Ministry of Forests *Seed Handling Guidebook* (Kolotelo and others 2001). Operational practices incorporate a Trimaco Supertuff 1.0 to 5.0 gal (3.8 to 18.9 L) paint strainer bag for imbibition and stratification of seedlots up to 2 lb (900 gr) in weight. For this treatment the representative 400-seed samples from each seedlot were placed into individual 100 percent nylon Organza 4 by 6 in (10 by 15 cm) mesh bags, tied off at the top, and subjected to a 14-day full-immersion, running-water rinse alongside operational greenhouse seedlots. The study plan for the operational-based treatment included a 10-minute soak in a 2:3 solution of 5.25 percent household bleach: water, followed by a 10-minute water rinse before the 14-day running-water rinse, to follow operational Webster Greenhouse practices. This step was inadvertently left out of all operational-based treatments in our trial, creating a significant variable between operational-based treatments and actual operational stratification. Research has shown that running-water treatments and pre-stratification bleach treatments can reduce surface-borne pathogens and increase germination capacity in some pines (*Pinus* spp.) (Axelrood and others 1995, Kolotelo and others 2001). Bleach treatments are specifically recommended for both high-value seed orchard seeds and high-risk species like WWP (Campbell and Landis 1990). Some growers prefer a 3-percent hydrogen peroxide solution for its reduced occupational-worker risk. For both of these chemicals, a post-immersion water rinse is important to minimize damage to seeds (Kolotelo and others 2001).

Following the running-water rinse, the seeds in the mesh bags were dried to a moisture content of 45 percent as measured by a Steinlite model 400G moisture meter, and the filled mesh bags were placed into a cooler at 35 °F (1 to 2 °C) for 30 days of stratification. During this period, the mesh bags were manipulated weekly to ensure uniform moisture content and were visually monitored for the presence of pathogens (Landis and others 1998). Following the 30-day period, the seed was removed from the mesh bags, dried to 35 percent moisture content, returned to the mesh bags, then stratified at 35 °F for an additional 90 days. One strategy behind this dual moisture content approach is to maintain sufficient surface moisture during the first stratification period to promote dormancy release, then to reduce moisture content during the second stratification period to minimize the spread of seedborne pathogens (Kolotelo and others 2001).

It proved difficult to maintain the moisture content at the desired 45 percent throughout the initial 30-day stratification period for this protocol, This problem is found to a lesser degree with the greenhouse's operational stratification, and reflects one of the characteristics observed by Kolotelo (2001)—the difficulty in producing equivalent results and conditions with both small and large quantities of seed.

At the end of the 120-day stratification period, the 400-seed samples for each of the 8 seedlots were removed from 35 °F (1 to 2 °C) conditions, a moisture content measurement was taken, and each sample was split into eight 50-seed replications. Each replication was spread evenly onto Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, and labeled by treatment before placement in a germination chamber.

Treatment 3—Low Moisture Operational-Based

The Low Moisture Operational-based lab protocol was developed as a simplified version of WA DNR Webster Nursery's stratification process. This treatment explores the lower end of the recommended range of moisture contents described by Edwards (1982) for removal of physiological dormancy with a goal of creating a condition less favorable for pathogen growth during the long stratification. The 8 representative 400-seed samples for this treatment were placed into individual 100 percent nylon Organza 4 by 6 in (10 by 15 cm) mesh bags, tied off at the top, and subjected to a 14-day full-immersion running-water rinse alongside operational greenhouse seedlots. As with treatment 2, the bleach treatment was in the study plan but was inadvertently left out of treatment 3.

Following the running-water rinse, the seed was surface dried in the mesh bags to a moisture content of 30 percent, as measured by a Steinlite model 400G moisture meter, and the mesh bags containing the seed were placed into stratification at 35 °F (1 to 2 °C) for 120 days. During this period, the mesh bags were not manipulated or closely monitored for the presence of pathogens.

At the end of the 120-day stratification period, the 400-seed samples for each of the 8 seedlots were removed from 35 °F (1 to 2 °C) conditions, a moisture content measurement was taken, and each sample was split into eight 50-seed replications. Each replication was spread evenly on Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, and labeled by treatment before placement in a germination chamber.

Germination Measurements

Germination trays for each treatment were placed into a Hoffman SG30 germination chamber, in which they were subjected to alternating cycles of 86 °F (30 °C) under lighted conditions for 8 hours and 68 °F (20 °C) under darkened conditions for 16 hours. Four germination counts were performed at 7-day intervals with the first at day 7 (figure 2). Each count included tallying and removing germinants whose radicle exceeded four times the length of their seedcoat. During these counts, abnormal germinants were recorded and discarded. At the end of the 4-week evaluation period, both the rate of germination and the cumulative germination capacity were calculated.

Assessment of Seedborne Pathogens

The long stratification required by WWP provides conditions favorable to the development and spread of several seedborne pathogens. Of these, *Fusarium* spp. and *Caloscypha fulgens* are often the most damaging. Seed moisture in excess of that needed for stratification can increase the growth of seedborne

Figure 2. Germination tray showing germinants for Seedlot 643 at day 7. From left to right: Treatments 1, 2, and 3. (Photos by Sheree Pickens, 2012)

pathogens, which is why surface drying is so important (Kolotelo and others 2001). *C. fulgens* can spread rapidly from diseased to healthy seeds during stratification, killing them before they have an opportunity to germinate. It can also infect adjacent seeds in multiple-sown cavities (Kolotelo and others 2001, Kolotelo 2013). *Fusarium* can also spread through contaminated seedlots during imbibition and stratification (Axelrood and others 1995, Kolotelo and others 2001).

Levels of pathogen infestation in the germination trays were observed and noted, but no attempt was made to identify specific fungi. The characterization of fungi was intended solely as a comparison of moisture contents and pre-stratification rinsing methods among the treatments. A useful description for identifying specific seedborne fungi can be found in Campbell and Landis (1990).

Statistical Analyses

Analysis of variance (ANOVA) was used to test the statistical significance of differences among treatments, seedlots, and the interaction between treatment and seedlot. The response variables for the ANOVAs were the number of seeds that had germinated by day 7 (initial germination) and by day 28 (final germination) (Neter and others 1996). Because most germination was expressed by day 14 for all seedlots, values for day 28 were considered to be representative of days 14 and 21 for the purposes of this analysis. Means for different treatments and seedlots were compared using linear contrasts and the error rate was controlled using the Tukey Honestly Significant Difference method (Neter and others 1996). ANOVA model terms were considered statistically significant at $\alpha < 0.05$. Analyses were conducted with the R statistical language and with the lsmeans package (Lenth 2013).

Results

Germination Rates and Capacities

The effects of stratification treatment, seedlot, and the treatment by seedlot interactions were all statistically significant for both initial and final germination (table 1, figure 3). Both treatment 2 (Webster Operational-based treatment) and treatment 3 (Low Moisture Operational-based treatment) had significantly greater initial germination rates than treatment 1 (AOSA standard treatment) for all seedlots. Initial germination was greater in treatment 3 than in treatment 2 in most cases, but differences among these treatments were only significant for Seedlot 1215. Treatments 2 and 3 also tended to have greater final germination than the AOSA standard treatment; however, the differences were not statistically significant for all of the seedlots (figure 3).

Pathogen Levels

As mentioned previously, an intended bleach treatment was not applied, so pathogen levels were potentially higher than they might have been. Observations of fungal infestations were made at a treatment level, rather than at a seedlot

Table 1. Summary of analysis-of-variance results testing the effects of stratification treatment, seedlot, and treatment by seedlot interactions on the number of initial germinants and final germinants of eight seedlots of western white pine.

Effect	DF	Initial germination (day 7)		Final germination (day 28)	
		F-Value	P-Value	F-Value	P-Value
Treatment	7	50.6	< 0.001	47.7	< 0.001
Seedlot	2	426.8	< 0.001	95.2	< 0.001
Treatment by seedlot	14	6.3	< 0.001	9.4	< 0.001
Error	168				

DF = degrees of freedom

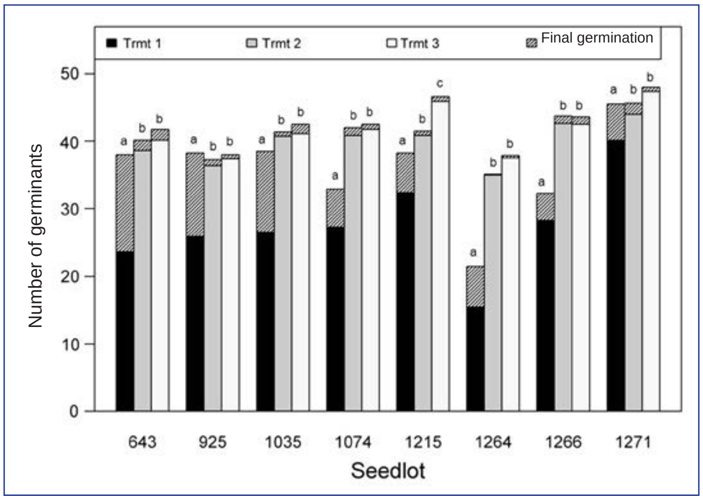

Figure 3. Germination capacity of eight seedlots for each of the three stratification treatments. Solid filled bars indicate germination capacity at day 7 (initial germination) for each treatment and diagonal patterned bars show germination capacity for each treatment at day 28 (final germination). Bars with different letters had significantly different germination capacity at day 7. (Peter Gould, 2013)

level, and were not quantified, so no patterns within seedlots could be established. For the AOSA Standard treatment, considerable fungi were present in the germination trays at the end of the 90-day stratification period. This condition was probably largely because of the absence of a running-water rinse in this treatment to cleanse the seedcoat (Axelrood and others 1995) and the lack of air exchange in the germination trays during the cold stratification and subsequent warm germination period. The Webster Operational-based treatment had a level of fungus in the germination trays less than that of the AOSA Standard treatment but still considerable, possibly because of the initial higher moisture stratification period, which provided favorable conditions for the spread of pathogens (Sutherland 1981, Cram and Fraedrich 2009). No fungi were present on seeds stratified with the Low Moisture Operational-based treatment, which was somewhat surprising given the largely unmonitored 120-day stratification period.

Discussion

Treatments 2 and 3, which included a 14-day rinse rather than a 24-hour soak, had better germination rates, better germination capacities, and lower pathogen levels. This supports research that such extended stratification treatments promoting thorough seed imbibition, the flushing of growth inhibitors from the seed coat and other structures, and the breaking of physical dormancy significantly improve germination (Kolotelo 1993; Bewley and Black 1994; Landis and others 1998; Bower and others 2011).

Higher germination rates and fewer seeds-per-cell sown as a result of higher germination capacities reduce initial greenhouse crop variation and accelerate germinant emergence. Benefits include higher overall crop quality, more complete genetic expression, and better seed use efficiency (Kolotelo and others 2001).

Seedlot 1264 is a specific example of the potential benefits of these alternate stratification approaches. Morphological observations made when the cones for this seedlot were initially received suggested that the collection was made before the seeds were mature. The initial lab test following collection returned a germination capacity of 31 percent, and a retest 1 year later showed that the germination capacity had increased to 45 percent—still a very poor result. The AOSA Standard protocol used in this study resulted in a germination capacity of 43 percent, whereas the Webster Operational-based and the Low Moisture Operational-based protocols resulted in germination capacities of 70 and 76 percent, respectively (figure 4). In this case, a seedlot that might have been designated for disposal was shown to be suitable for operational sowing when using an alternative stratification protocol.

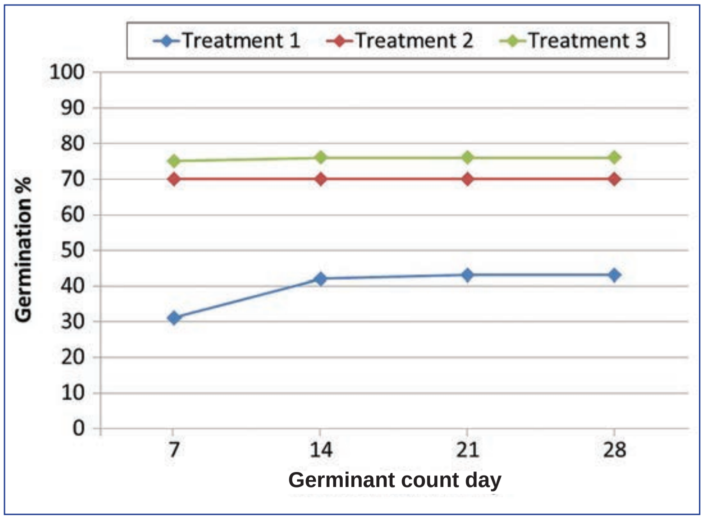

Figure 4. Cumulative germination percentage over time for Seedlot 1264, showing improved performance of alternative stratification treatments for this seedlot collected early in the season. (Sheree Pickens, 2012)

Potential seed savings as a result of the Webster and Low Moisture Operational-based alternatives are also illustrated in Seedlot 1074. Kolotelo and others (2001) describe the use of a “125 Percent Green Tree Count” to ensure that container seedling orders are fulfilled and that the probability of a viable seedling in each cavity is close to 100 percent. As part of this approach, the publication includes a table, which describes the number of seeds per cavity that must be sown to attain this goal. We used this table to determine values for Seedlot 1074 (table 2). At the AOSA Standard protocol, the germination capacity of 66 percent for Seedlot 1074 requires a sowing rate of 5.22 seeds per cavity to meet the 125 percent green tree count. Germination capacities of this same seedlot for the Webster Operational-based and Low Moisture Operational-based protocols were 84 and 85 percent, respectively, which would require sowing rates of 3.43 and 3.30 seeds, respectively, per cavity to meet the 125 percent green tree count—a 35-percent reduction in seed needed compared with the AOSA Standard protocol.

Table 2. Cumulative germination over time for Seedlot 1074, demonstrating improved seed use efficiency of alternative stratification treatments. The “125 Percent Green Tree Count” refers to a sowing approach that produces 25 percent extra seedlings beyond the requested amount from which to select shippable trees (Kolotelo and others, 2001).

Seedlot	Treatment	Germination capacity (%)	Seeds to sow per cavity to achieve 125% Green Tree Count
1074	1	66	5.22
1074	2	84	3.43
1074	3	85	3.30

Lower WWP sowing rates as a result of optimized lab germination tests not only result in seed savings, but they lead to better expression of blister rust-resistant families, as the amount of thinning is reduced (Landis and others 1998; El-Kassaby 2000). Single seed or individual family sowing would be ideal to ensure full expression of desirable traits (El-Kassaby and Thomson 1996), but consistent WWP germination capacities to make this approach economically feasible have not yet been realized.

Pros and Cons of the Webster Operational-Based Treatment Versus the Low Moisture Operational-Based Protocol

Although the Low Moisture Operational-based protocol (treatment 3) returned slightly higher germination capacity and lower pathogen levels, drawbacks to using this protocol could exist. The moisture level used in this protocol was at the low end of the range recommended for dormancy release, so it is possible that families within particular seedlots may not have full-dormancy release, resulting in overall germination performance poorer than that of the Webster Operational-based treatment (treatment 2). This possibility should be weighed against the absence of pathogens seen in the Low Moisture Operational-based treatment.

Implications for Future Practices at Webster Nursery

The most obvious drawback of this trial was the fact that no sanitation rinse was used in the treatments. Based on past research, it can be assumed that the addition of a bleach rinse to the stratification process would result in lower pathogen levels and possibly even higher germination capacities as well. A similar earlier version of the trial supports this assumption. In the earlier trial, a bleach rinse applied to five of the same seedlots resulted in average germination counts 13 and 17 percent higher for the AOSA Standard treatment and the Webster Operational-based treatment, respectively, at day 7 compared with the later bleach-free trial described here. For all of the seedlots tested in the previous trial with the bleach rinse, the Webster Operational-based treatment resulted in germination capacities in excess of 94 percent, which approaches an efficient one-seed-per-cavity sowing.

In addition to ensuring that a bleach rinse is incorporated, subsequent modifications will likely include monitoring of water temperatures, further efforts to address the difference between a successful lab germinant and a successful greenhouse germinant, more detailed fungal assays, and closer tracking of moisture contents throughout the stratification process. As recommended by Kolotelo and others (2001), quantifying and evaluating heat sums of different stratification protocols as they relate to germination speed may also be useful. In addition, we plan to compare the results described in this article with future trials with seeds from the same seedlots sown into an operational greenhouse setting for a real-world comparison.

We encourage other greenhouse nurseries that work closely with seed testing facilities to conduct similar trials of their own to evaluate and compare WWP stratification practices. The sharing and refinement of these results over time could eventually lead to modifications in official seed testing rules to more closely mirror operational practices.

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Conserved *Ex Situ* Genetic Resources of Eastern and Carolina Hemlock: Eastern North American Conifers Threatened by the Hemlock Woolly Adelgid

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Abstract

The long-term sustainability of the eastern North American conifers eastern hemlock (*Tsuga canadensis* [L.] Carriére) and Carolina hemlock (*T. caroliniana* Engelmann) is threatened by the exotic insect hemlock woolly adelgid (*Adelges tsugae* Annand; HWA). The integrated pest management strategy to mitigate HWA impacts on hemlock ecosystems includes a cooperative genetic resource conservation program being conducted by Camcore (International Tree Breeding and Conservation Program at North Carolina [NC] State University) and the U.S. Department of Agriculture (USDA) Forest Service Forest Health Protection. Through the first 10 years of this project (2003 to 2013), seeds have been collected from 60 populations of eastern hemlock and 19 populations of Carolina hemlock in the United States, representing 451 and 134 mother trees, respectively. Seeds have been distributed to the Camcore seed bank in Raleigh, NC, and the USDA Agricultural Research Service National Center for Genetic Resource Preservation in Fort Collins, CO, for long-term storage, and to forest nurseries in Brazil, Chile, and the United States, where seed orchards have been established.

Introduction

Hemlocks (*Tsuga* Carriére) are long-lived conifers that are among the most shade-tolerant and drought-susceptible species in the Pinaceae family, with some of the oldest recorded specimens surviving for 800 to 1,000 years. Worldwide distribution is restricted to three geographic regions (Farjon 1990) for the nine taxonomically accepted hemlock species. Five species occur in eastern Asia, distributed throughout mainland China, the Himalayan Mountains, and Taiwan (Chinese hemlock [*T. chinensis* (Franc.) Pritzel in Diels], Himalayan hemlock [*T. dumosa* (D. Don) Eichler], and Forrest's hemlock [*T. forrestii* Downie]) and in Japan (southern Japanese hemlock [*T. sieboldii* Carriére] and northern Japanese hemlock [*T. diversifolia* (Maxim.) Masters]). Four species occur in North America. Western hemlock (*T. heterophylla* [Raf.] Sargent) and mountain hemlock (*T. mertensiana* [Bong.] Carriére) occur in western North America in a range that extends from southern Alaska south into northern California. Eastern hemlock (*T. canadensis* [L.] Carriére) and Carolina hemlock (*T. caroliniana* Engelmann) are native to eastern North America; they are the subjects of this article.

Eastern hemlock is a widespread conifer species with a natural range that extends from Nova Scotia west to northern Minnesota, south throughout the New England and Middle Atlantic States, and down the southern Appalachian Mountains into northern Georgia and the Cumberland Plateau of Alabama (Farjon 1990). A number of disjunct populations occur to the west of the main distribution in Alabama, Tennessee, Kentucky, Indiana, Ohio, Wisconsin, and Minnesota and to the east in Maryland, Virginia, and North Carolina (figure 1). The species is found from sea level to 4,920 ft (1,500 m) elevation and

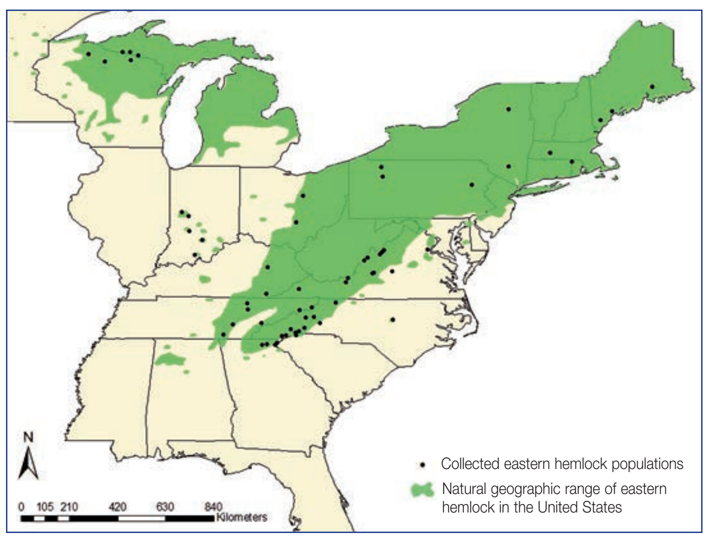

Figure 1. The geographic distribution of eastern hemlock (*Tsuga canadensis*) in the Eastern United States and the locations of provenance seed collections made by Camcore. (Shapefile based on Little 1971; downloaded from U.S. Geological Survey 2013)

is bimodal in habitat distribution (Kessell 1979). It occurs in high abundance on moist, well-drained, nutrient rich soils of mesic riparian zones and seasonably moist subxeric areas. At the higher end of its elevational range, eastern hemlock is often more scattered in occurrence along exposed xerophytic slopes and ridges. Where it occurs as a riparian species, the tree is important for soil stabilization and water quality and serves as a haven for associated aquatic species and as winter shelter for white-tailed deer (*Odocoileus virginianus* Zimm.), ruffed grouse (*Bonasa umbellus* L.), turkey (*Meleagris gallopavo* L.), and several other species (Ellison and others 2005).

Carolina hemlock, first described in 1837 on Table Rock Mountain in South Carolina (James 1959), is a southern Appalachian endemic with a patchy distribution occurring throughout the mountain and upper Piedmont regions of Virginia, Tennessee, Georgia, North Carolina, and South Carolina (Farjon 1990; figure 2). The geographic range is small, approximately 289 mi by 102 mi (465 km by 165 km) with a latitudinal range from 37°40’ N. in Rockbridge County, VA, south to 34°73’ N. in Rabun County, GA (Jetton and others 2008a). Carolina hemlock populations have been reported as far north of the range as northeastern Ohio, where a small occurrence of the species is found at the Richie Ledges overlook in the Cuyahoga Valley National Park (Galehouse 2007). Although some researchers believe the occurrence to be natural, other researchers suggest the trees were likely planted by the Civilian Conservation Corps in the 1930s. Unlike eastern hemlock, Carolina hemlock is most abundant along dry, north-facing, rocky ridge tops at elevations of 1,960

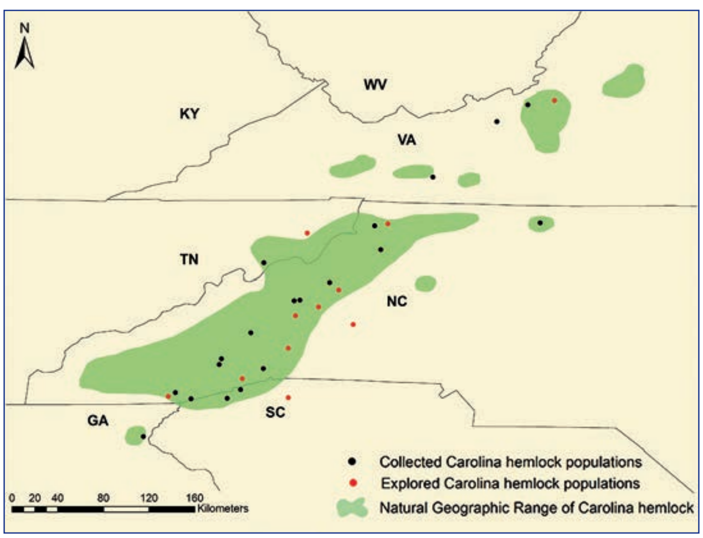

Figure 2. The geographic distribution of Carolina hemlock (*Tsuga caroliniana*) in the southern Appalachian Mountains and the locations of provenance seed collections made by Camcore indicated by black circles. Red circles indicate provenances where seed collections have not yet been made. (Shapefile based on Little 1971; downloaded from U.S. Geological Survey 2013)

to 4,900 ft (600 to 1,500 m) (Humphrey 1989). Preferred soils are dry, acidic, and nutrient poor, although more recent studies indicate the species to be more broadly adapted to a variety of soil types than initially thought (Jetton and others 2008a). Scattered populations occasionally are found growing in mesic or riparian settings more typical of eastern hemlock (James 1959). In its typical habitat, Carolina hemlock helps to reduce soil erosion while providing forage and shelter for white-tailed deer (Rentch and others 2000). Carolina hemlock is also highly regarded for its rugged aesthetic beauty and is commonly used in the ornamental industry (Swartley 1984).

Despite the differences in distribution and habitat between the two species, the long-term sustainability of both eastern and Carolina hemlock faces a significant threat because of the hemlock woolly adelgid (*Adelges tsugae* Annand; HWA). The HWA is an exotic insect introduced to eastern North America from Japan sometime before the mid-1950s and has caused widespread decline and mortality across 100 percent of the Carolina hemlock range and approximately 50 percent of the eastern hemlock range (McClure and others 2001, USDA Forest Service 2011). The integrated strategy to mitigate the impacts of HWA on hemlock ecosystems involves both *in situ* and *ex situ* approaches to hemlock conservation. The *in situ* approaches that have received the most attention are chemical control with systemic insecticides and biological control through the importation, rearing, and release of predators from the native range of HWA. Both show much promise, but the use of insecticides is limited in scope by logistical and ecological concerns, while biological control requires a decade or more of additional research and development before widespread effectiveness is realized. A complementary approach to these *in situ* efforts is *ex situ* genetic resource conservation, where genetically representative seed samples are collected from natural stands distributed across the range of eastern and Carolina hemlock. Seeds are placed either into seed banks for long-term storage or are used to establish seed orchards in areas where HWA is unlikely to occur or where the trees can be effectively protected from the insect with insecticides. After being established, these strategic seed reserves and seed orchards can serve as a source of highly diverse and broadly adaptable genetic material for restoration and reforestation when *in situ* HWA management strategies become more broadly effective.

This article reports on progress made through the first 10 years (2003 to 2013) of a cooperative hemlock genetic resource conservation program being conducted by Camcore (International Tree Breeding and Conservation Program at NC State University) and the USDA Forest Service, Forest

Health Protection program. The article also reviews the distribution, biology, and management of HWA. Progress at earlier stages of this project was documented by Tighe and others (2005), Jetton and others (2008a,b), and Jetton and others (2010).

Hemlock Woolly Adelgid

Worldwide Distribution

The worldwide distribution of HWA mirrors that of the hemlocks, with three primary concentrations found in eastern Asia, western North America, and eastern North America (Havill and others 2006). HWA is native to Asia, where it is widespread and can be found feeding on all five hemlock species native to the region but is largely innocuous because of a combination of evolved host resistance and predation by natural enemies (McClure and others 2001). The first scientific descriptions of the insect are from northwestern North America, where HWA occupies a range extending from southern Alaska to northern California. These initial reports were based on specimens collected from western hemlock in California and Oregon, where it was initially thought that HWA was exotic (Annand 1924) but is now known to be endemic (Havill and others 2011). HWA is an exotic pest in eastern North America, where little evidence exists of natural host resistance and where no native natural enemies are capable of limiting adelgid population growth (Wallace and Hain 2000; McClure and others 2001). It was introduced into Richmond, VA, sometime before the mid-1950s (Havill and others 2006), most likely on ornamental hemlock nursery stock imported from Japan. Although the initial spread of HWA from its point of origin was rather slow, its distribution in the Eastern United States has expanded rapidly since the mid-1980s and now covers a 19-State area, ranging from southern Maine south along the Appalachian Mountain chain to northern Georgia and west into Ohio (USDA Forest Service 2011).

Life History and Host Impacts

HWA has a complex life cycle that includes multiple generations and life forms per year that alternate between hemlock and spruce (*Picea* spp.) hosts using a combination of sexual and parthenogenetic reproductive strategies. For additional details on HWA's life history and how its timing differs with latitude, readers are referred to McClure (1989), Gray and Salom (1996), Havill and Foottit (2007), and Joseph and others (2011). The following refers to those parts of the HWA life cycle that occur on hemlock in the Eastern United States. The two parthenogenetic generations of HWA that are damaging to hemlocks are known as the sistens and progrediens and are active on the tree from October through early July (McClure 1989). They are identified by the cottony white woolly masses from which the insect derives its common name. These woolly masses or "ovisacs" can be observed at the base of needles on the underside of infested hemlock branches (figure 3). Each ovisac contains a single adult female and her parthenogenetically produced eggs. HWA crawlers (first instar nymphs), the only mobile life stage, hatch from the eggs and settle at feeding sites at the base of hemlock needles, where the insects remain for the remainder of their life. Settlement occurs either on the natal tree or on a nearby tree after wind, bird, deer, or human mediated passive dispersal (McClure 1990). HWA feeds by inserting its piercing-sucking mouthparts into the

Figure 3. White woolly ovisacs of the hemlock woolly adelgid (HWA) on eastern hemlock (left). HWA caused eastern hemlock mortality in the Shenandoah National Park, VA (right). (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

needle cushion and extracting stored nutrients from xylem ray parenchyma cells (Young and others 1995). Under high HWA population densities, continued depletion of nutrient stores leads to needle desiccation and drop, abortion of vegetative and reproductive buds, and cessation of new growth. Severe infestations can kill trees in as little as 4 years, although some individual trees have persisted for 10 to 20 years before succumbing to HWA. A high lifetime fecundity rate of up to 300 viable eggs per female (McClure and others 2001) has likely attributed to HWA's rapid spread and widespread impacts despite its otherwise limiting life history strategies of parthenogenesis and passive dispersal.

Integrated Pest Management

The integrated pest management strategy for managing HWA damage to eastern North American hemlock forests is focused in the areas of chemical control, biological control, host resistance breeding, and gene conservation. The chemical insecticides imidacloprid (applied by soil drench, soil injection, or stem injection) and dinotefuran (applied as a basal trunk spray) are currently the only truly effective methods to control HWA and retain hemlock in a forest or landscape (Vose and others 2013). Single applications of each chemical can effectively control HWA for 3 or more years. However, due to logistic, economic, and ecological concerns, the use of these chemicals is limited to individual high-value trees or small groups of trees in ornamental and recreation settings or the more easily accessible woodland areas of forests and parks. Lower cost and less toxic high pressure foliar sprays of insecticidal soaps and horticultural oils are also available for HWA control, but lack extended efficacy and need to be reapplied annually to maintain control (Vose and others 2013).

Biological control through the importation and release of HWA predators from the native range of HWA is currently considered the best long-term solution to management of the pest in forest settings (Onken and Reardon 2011). Several species of predatory beetles (Coleoptera) in the genera *Sasajiscymnus*, *Scymnus*, and *Laricobius* and predatory flies (Diptera) in the genus *Leucopis* are currently in various stages of laboratory study, field efficacy trials, mass rearing, and widespread release. *Sasajiscymnus tsugae* (Sasaji and McClure) has been most widely distributed across the Eastern United States with close to 3 million adult beetles released to date. However, good estimates of establishment and field impact on HWA populations are generally lacking other than at intensively sampled release sites in Connecticut (Cheah 2011). *Laricobius nigrinus* Fender and *L. osakensis* Montgomery and Shiyake appear to be the most promising predators currently under evaluation, having demonstrated successful establishment and impact on HWA population density in the field (*L. nigrinus*; Mausel and others 2008) and the ability to respond in number and function to HWA density in laboratory evaluations (*L. osakensis*; Vieira and others 2012). The ultimate goal of this program is to establish a suite of natural enemies whose feeding and impact will combine to regulate HWA populations below damaging levels (Vose and others 2013). For more detailed information on the effort to implement HWA biological control in eastern North America, see Onken and Reardon (2011).

Host resistance breeding as a strategy to mitigate the impacts of HWA has received relatively little attention compared with other management options, but progress has been made in this field during the past 10 years. Most research has focused on understanding the host-insect interaction between hemlocks and HWA, and determining how this differs between resistant and susceptible hemlock genotypes. Plant characteristics evaluated include variation in foliar terpenoid, nutrient, amino acid, and wax chemistries (Lagalante and Montgomery 2003, Pontius and others 2006, Gomez and others 2012); changes in water conductivity and formation of false rings (Gonda-King and others 2012, Domec and others 2013); and the presence and severity of hemlock hypersensitive responses to HWA attack (Radville and others 2011). Emphasis has also been placed on the production of hybrids between HWA-susceptible eastern and Carolina hemlocks and resistant species from Asia and western North America. One goal of the hybridization program is to eventually establish a backcross breeding program similar to what has been accomplished for American chestnut (*Castanea dentate* [Marsh.] Borkh.) for reintroducing HWA-resistant hemlock genotypes into heavily impacted areas. Thus far, eastern hemlock has shown a high level of hybrid incompatibility with other hemlock species, but a number of successful crosses between Carolina hemlock and Chinese hemlock have been produced (Bentz and others 2002). Finally, although both eastern and Carolina hemlock were initially thought to be universally susceptible to HWA, some evidence suggests that natural HWA resistance might exist in both species (Caswell and others 2008; Jetton and others 2008c). More research is needed to verify the validity of these assertions, but, if verified and the level of genetic variation for such traits is adequate, it suggests the possibility of breeding and restoration programs based on the pure species rather than sole dependence on the use of genotypes that contain some proportion of genes from nonnative hemlocks.

Hemlock Genetic Resource Conservation

Genetic Resource Conservation Rationale and Objectives

Because chemical insecticides are limited in use, biological control requires additional years of research and development before reaching anticipated levels of efficacy, and hemlock decline and mortality in the Eastern United States continues unabated, an effort to conserve gene pools of eastern and Carolina hemlock being lost to HWA is critical to the long-term sustainability of these ecologically vital species. The primary objective of the Camcore/USDA Forest Service cooperative hemlock genetic resource conservation program is to maintain, in perpetuity, viable *ex situ* seed reserves and seedling seed orchards of both species that will serve as a source of genetic material for breeding and restoration activities once effective *in situ* hemlock conservation strategies are in place. Another way to view this effort is as an insurance policy against the "worst case scenario," where both eastern and Carolina hemlocks are functionally eliminated by HWA from the forest ecosystems of eastern North America.

The conservation program was initiated in 2003 and was designed to include four phases. Phase 1 (2003 to 2005) focused on seed collections from stands of Carolina hemlock throughout its southern Appalachian range in Georgia, North Carolina, South Carolina, Tennessee, and Virginia. Phase 2 (2005 to 2009) focused on seed collections from stands of eastern hemlock in the southern portion of its range. The southern range was defined as Alabama, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, and Virginia, where eastern hemlock occurs. Phase 3 (2009 to 2012) focused on seed collections across eastern hemlock's northern range and included Connecticut, Delaware, Indiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, West Virginia, and Wisconsin. Phase 4 is currently under way and is focused on establishing conservation seed orchards inside and outside the United States and additional seed collections, where needed, in the three regions described previously.

Given the much larger geographic distribution of eastern hemlock compared with Carolina hemlock, the seed collection effort for the species was split among the USDA Forest Service Southern and Eastern Regions to make collection planning and implementation logistically easier. The initial focus was on the Southern Region because of the much higher rates of HWA-related decline and mortality that have occurred among hemlock populations in the region. HWA impacts have been less severe in the Eastern Region (northern range of eastern hemlock), and, even now, large areas remain HWA free (USDA Forest Service 2011).

Seed Collection Strategy and Protocols

Common questions associated with the beginning of a new gene conservation program are how many populations and mother trees per population to sample, and how to distribute seed collections across the range of a species to capture maximum levels of diversity and broad adaptability. A good understanding of species population genetic structure and environmental adaptability are key to answering these questions and designing gene conservation strategies that are effective at capturing a representative number of alleles. From lessons learned during 32 years of research focused on the conservation and testing of pine species native to the fragmented tropical and subtropical forests of Mexico and Central America (Dvorak and others 2000), Camcore has determined that seed collections from 10 to 20 trees per population, depending on population size, will capture most alleles that occur at frequencies of 5 percent or greater, assuming low to moderate levels of genetic diversity (Dvorak and others 1999). Sampling 6 to 10 populations distributed across the geographic range of a species is necessary to also capture broad environmental adaptability.

Before the beginning of this project, no research on the population genetic structure of Carolina hemlock had been conducted. Therefore, as part of the effort to design the seed sampling strategy for the species, Camcore conducted an amplified fragment length polymorphism (AFLP) molecular marker analysis of 15 Carolina hemlock populations (Potter and others 2010). This study indicated that the species has a moderate level of genetic diversity (He = 0.302) for a conifer, and, because most Carolina hemlock populations are relatively small (Jetton and others 2008a), indicates that a sample of up to 10 trees per population should be sufficient to obtain a genetically representative seed sample. However, the results also indicated a high level of genetic differentiation exists among the populations, likely because of their isolated nature. Furthermore, Carolina hemlock is adapted to a number of soil types (Jetton and others 2008a) and appears to have moderately broad climatic adaptability in a range that extends across five (5b to 7b) plant hardiness zones (USDA Agricultural Research Service 2012). These factors, together with the fact that Carolina hemlock has been identified as the tree species most at risk for genetic degradation because of climate change (Erickson and others 2012), suggest that seed should be sampled from a larger than typical number of populations to adequately capture the diversity and adaptability present in the species. Therefore, to protect against the loss of the species to both HWA and climate change, the seed collection strategy that has been adopted for Carolina hemlock is to sample up to 10 mother trees per population in as many populations as can be identified. This strategy includes an effort to sample locations within each of the five plant hardiness zones occupied by the species.

Similar to the situation with Carolina hemlock, at the outset relatively little data existed on population genetic structure and diversity in eastern hemlock that was useful for designing a gene conservation strategy focused on the entire range of the species. One small study, an isozyme diversity analysis, had been completed and found that eastern hemlock has an extremely low level of genetic diversity (Zabinski 1992). The results suggest that seed collection intensity could be relatively low (i.e., few populations and few trees per population) and still be genetically representative. Zabinski's study focused primarily on eastern hemlock populations in the Lake States region, however, and thus was not sufficient for basing sampling decisions in other parts of the species' range.

To expand on the available data, Camcore conducted two studies on the population genetics of eastern hemlock: one that used isozymes to evaluate 20 populations in the southern Appalachian region (Potter and others 2008), and a second that used microsatellite molecular markers to assess genetic structure in 60 populations distributed across the entire range of the species (Potter and others 2012). As the gene conservation program for eastern hemlock has developed, the results of the latter study have been the most useful to the design of the seed collection strategy and fit nicely with the two-region approach to sampling. The microsatellite data revealed two pockets of high genetic diversity for eastern hemlock where higher seed sampling intensity is necessary. One pocket is located in the Blue Ridge Mountains of the southern Appalachian region, and the second is in New York and southern New England. Diversity was low to moderate outside of these pockets, especially in the disjunct populations that occur to the east and west of the main eastern hemlock distribution. Although disjunct populations are expected to have low diversity, Potter and others (2012) found that many of these harbor a high number of rare alleles that do not occur elsewhere in the range, making them important targets for seed collection. Eastern hemlock also has broad climatic adaptability across its large geographic distribution in the United States that extends across 10 (3a to 7b) plant hardiness zones (USDA Agricultural Research Service 2012).

Based on the patterns of genetic structure and climatic adaptability described previously, it was determined that seed collections targeting 10 mother trees per population across 30 populations in both the southern and northern ranges would be sufficient to obtain representative seed samples of eastern hemlock. Emphasis in the collection work is being placed in the pockets of high genetic diversity and disjunct populations that occur in each region, with a lower sampling intensity in lower diversity portions of the main species distribution. As with Carolina hemlock, an effort to sample all plant hardiness zones occupied by eastern hemlock is being made. This strategy should yield collections from up to 600 mother trees and 60 populations.

During seed collections for both species, a distance of 160 to 320 ft (50 to 100 m) is maintained between selected trees within individual populations as a buffer against relatedness. All trees sampled are tagged with a unique pedigree number, and height (m), diameter (cm), elevation (m), geographic coordinates, and presence/absence of HWA is recorded. A detailed description of the site selection and seed collection protocols for this project is available in Jetton and others (2007).

Provenance Seed Collections

Between 2003 and 2010, Camcore collected seed from 134 mother trees in 19 populations that were well distributed across the range of Carolina hemlock (table 1, figures 2 and 4). Although 11 additional populations have been identified and explored, seed collections have not yet been completed in those locations because of recurrence of poor cone crops. Where collections were conducted, an average of 7 mother trees per population were sampled, ranging from as few as 1 (Upper Whitewater Falls and Whiteside Mountain) to as many as 12 (Cliff Ridge and Hanging Rock). Total seed yield from these 19 populations was 1,515 g (53 oz) (table 1). At an estimated 360 seeds per gram (Barbour and others 2008), this totals more than 500,000 Carolina hemlock seeds placed into conservation. An average of five viable seeds per cone were obtained. Based on an estimated seed potential per cone of 24 (Farjon 1990), seed efficiency in the Carolina hemlock populations sampled was 21 percent (seed efficiency = number of filled seeds/seed potential). Collections represent four of the five plant hardiness zones where the species occurs (table 1; zone 5b not yet sampled).

Table 1. Location, climate, seed collection, and viability data for Carolina hemlock (*Tsuga caroliniana*) provenances sampled for *ex situ* gene conservation.

Provenance	County, State	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[a] hardiness zone	Seed collection year	Mother[b] trees (#)	Total seeds (g)	Total[c] seed (#)	Seed[d] viability (%)
Biltmore Estate	Buncombe, NC	35.55	– 82.54	573	7a	2007	6	66.4	24,435	25.0
Bluff Mountain	Ashe, NC	36.39	– 80.25	1,420	6a	2003	8	23.9	8,795	11.3
Caesar's Head	Greenville, SC	35.10	– 82.62	920	7a	2003/2006	7	56.6	20,821	12.5
Carl Sandburg Home	Henderson, NC	35.27	– 82.44	682	7a	2009	6	83.5	30,728	25.5
Carolina Hemlocks Campground	Yancey, NC	35.80	– 82.20	880	6b	2003/2008	11	234.8	86,406	43.7
Cliff Ridge	Unicoi, TN	36.10	– 82.44	550	6b	2006/2008	12	151.1	55,605	29.3
Crabtree	Yancey, NC	35.81	– 82.16	1,170	6b	2003	6	43.8	16,100	11.2
Cradle of Forestry	Transylvania, NC	35.34	– 82.77	990	6b	2003/2008	11	89.2	32,826	26.2
Cripple Creek	Wythe, VA	36.77	– 81.11	740	6a	2006/2008	7	99.8	36,726	3.5
Hanging Rock	Stokes, NC	36.41	– 80.26	480	6b	2003/2009	12	128.8	47,398	1.3
Linville Falls	McDowell, NC	35.94	– 81.92	970	6a	2003	10	247.8	91,190	51.2
Looking Glass Rock	Transylvania, NC	35.30	– 82.79	1,179	7a	2010	8	36.2	13,322	0.5
New River	Montgomery, VA	37.21	– 80.60	556	6b	2009	6	113.6	41,805	5.5
Sinking Creek	Craig, VA	37.34	– 80.36	880	6a	2009	6	38.9	14,315	16.0
Table Rock	Pickens, SC	35.03	– 82.73	992	7a	2003	3	2.8	1,045	16.3
Tallulah Gorge	Rabun, GA	34.73	– 83.39	430	7b	2005	3	27.6	10,157	21.5
Upper Whitewater Falls	Jackson, NC	35.03	– 83.01	790	7a	2009	1	16.5	6,083	2.0
Whiteside Mountain	Jackson, NC	35.08	– 83.13	1,407	6b	2009	1	9.9	3,647	25.0
Wildcat	Watauga, NC	36.20	– 81.52	850	6b	2003	10	43.8	16,100	3.8

[a] Determined using interactive plant hardiness zone map available online: http://planthardiness.ars.usda.gov/PHZMWeb/InteractiveMap.aspx.

[b] Total number of mother trees per provenance from which seed was collected.

[c] Based on an average of 360 seeds/g (Barbour and others 2008).

[d] Based on 30-day Petri dish germination assays conducted at 22 °C, 16:8 L:D, and with two 50-seed replications per provenance.

Figure 4. Andy Whittier (Camcore) collecting seed cones from Carolina hemlock on Looking Glass Mountain, Pisgah National Forest, NC. (Photo courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

For eastern hemlock, Camcore collected seed from 451 mother trees in 60 populations distributed across the species' southern and northern ranges between 2005 and 2012 (table 2, figure 1). Most of these collections occurred in the southern range, where 270 trees in 37 populations were sampled. In the northern range, 181 mother trees in 23 populations have been sampled. Total seed yield across all 60 populations was 5,544 g (196 oz) (figure 5). At an estimated 412 seeds per gram (Barbour and others 2008), 5,544 g equates to more than 2 million seeds conserved. The number of mother trees sampled per population ranged from 1 (Whiteside Mountain) to 24 (Great Smoky Mountains National Park), with an average of 8. Seed efficiency in eastern hemlock was also 21 percent, based on an average of 6 viable seeds per cone and an estimated seed potential of 28 (Farjon 1990). Collections represent 7 of the 10 plant hardiness zones where the species occurs (table 2; zones 3a, 3b, and 4b not yet sampled).

Table 2. Location, climate, seed collection, and viability data for eastern hemlock (*Tsuga canadensis*) provenances sampled for *ex situ* gene conservation.

Provenance	County, State	Range[a]	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[b] hardiness zone	Seed collection year	Mother[c] trees (#)	Total seeds (g)	Total[d] seeds (#)	Seed[e] viability (%)
Arbutus Pond	Essex, NY	N	43.97	– 74.23	497	4a	2011	10	118.1	48,657	22.8
Big Walnut Nature Preserve	Putnam, IN	N	39.77	– 86.78	232	5b	2011	5	56.7	23,360	16.0
Bradbury State Park	Cumberland, ME	N	43.89	– 70.18	84	5b	2011	7	178.7	73,624	7.1
Cook Forest State Park	Forest, PA	N	41.32	– 79.18	357	5b	2009/2011	8	317.8	130,917	28.7
Echo Lake	Vilas, WI	N	45.91	– 89.04	525	4a	2010	10	43.6	17,963	37.0
George Washington National Forest	Providence, RI	N	41.93	– 71.75	207	6a	2011	9	24.8	10,218	9.1

Table 2. Location, climate, seed collection, and viability data for eastern hemlock (*Tsuga canadensis*) provenances sampled for *ex situ* gene conservation (continued).

Provenance	County, State	Range[a]	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[b] hardiness zone	Seed collection year	Mother[c] trees (#)	Total seeds (g)	Total[d] seeds (#)	Seed[e] viability (%)
Green's Bluff Nature Preserve	Owen, IN	N	39.20	– 86.76	175	6a	2011	3	10.9	4,491	0.0
Hearts Content Recreation Area	Carbon, PA	N	41.71	– 79.24	527	5b	2011	10	196.1	80,793	19.8
Hemlock Bluff Nature Preserve	Jackson, IN	N	38.84	– 86.26	190	6a	2011	5	7.6	3,131	0.5
Hemlock Cliffs	Crawford, IN	N	38.27	– 86.53	192	6a	2011	4	3.8	1,566	2.0
Hickory Run State Park	Carbon, PA	N	41.02	– 75.68	461	5b	2011	10	62.4	25,709	14.0
Hocking Hills State Park	Hocking, OH	N	39.55	– 82.57	324	5b	2011	6	24.1	9,929	17.5
Imp Lake	Gogebic, MI	N	46.22	– 89.07	537	4a	2011	10	49.3	20,312	25.9
Lake Ottawa	Iron, MI	N	46.08	– 88.75	500	4a	2011	8	47.8	19,694	22.5
Massabesic Experimental Forest	York, ME	N	43.56	– 70.64	64	5b	2011	7	279.5	115,154	16.5
Minnewaska State Park	Ulster, NY	N	41.73	– 74.23	426	6a	2011	10	152.2	62,706	14.2
Mohican Forest State Park	Ashland, OH	N	40.59	– 82.30	424	5b	2011	5	4.1	1,689	3.0
Mount Tom State Reservation	Hampden, MA	N	42.26	– 72.61	350	6a	2011	9	257.1	105,925	4.2
Muskellunge Creek	Ashland, WI	N	46.14	– 90.70	483	4a	2010	10	40.7	16,768	46.0
Penobscot Experimental Forest	Penobscot, ME	N	44.85	– 68.62	51	5a	2011	10	263.6	108,603	7.7
Pine Hills Nature Preserve	Parke, IN	N	39.94	– 87.05	176	5b	2011	5	35.9	14,791	0.5
Round Lake	Price, WI	N	45.84	– 90.07	501	4a	2010	10	75.2	30,987	59.0
Sylvania Wilderness	Gogebic, MI	N	46.23	– 89.36	542	4a	2011	10	6.8	2,802	31.0
Anna Ruby Falls	White, GA	S	34.76	– 83.71	708	7b	2010	2	11.3	4,651	13.0
Back Creek	Burke, NC	S	35.83	– 81.86	412	7b	2008	6	50.7	20,884	19.6
Beech Mountain	Avery, NC	S	36.22	– 81.94	987	6a	2006	5	35.4	14,577	12.9
Blowing Springs	Bath, VA	S	38.06	– 79.89	522	6a	2007	3	4.9	2,019	4.5
Braley Pond	Augusta, VA	S	38.28	– 79.29	614	6a	2009	3	7.9	3,255	6.5
Carl Sandburg Home	Henderson, NC	S	35.27	– 82.44	689	7a	2007	5	8.8	3,626	13.5
Carolina Hemlocks Campground	Yancey, NC	S	35.80	– 82.20	880	6b	2008	7	102.1	42,045	22.8
Cave Mountain Lake	Rockbridge, VA	S	37.57	– 79.53	370	7a	2007	3	3.8	1,566	7.0
Chattooga River	Oconee, SC	S	34.81	– 83.30	344	7b	2007	10	116.2	47,874	50.5
Cliff Ridge	Unicoi, TN	S	36.10	– 82.44	522	6b	2006/2008	10	113.7	46,832	21.5
Cradle of Forestry	Transylvania, NC	S	35.34	– 82.77	990	6b	2008	10	144.6	59,588	23.0
DuPont State Forest	Transylvania, NC	S	35.21	– 82.58	820	7a	2006/2007	10	19.1	7,869	11.4
Frozen Head State Park	Morgan, TN	S	36.14	– 84.47	557	6a	2008/2012	15	90.2	37,162	44.3
Great Smoky Mountains National Park	Blount, TN	S	35.61	– 83.93	427	6a	2008	24	1399.5	576,578	56.7
Guest River Gorge	Wise, VA	S	36.92	– 82.45	606	6b	2009	3	3.8	1,578	22.5
Helton Creek	Rabun, GA	S	34.75	– 83.89	731	6b	2007	4	54.1	22,297	21.5
Hemlock Bluffs Nature Preserve	Wake, NC	S	35.72	– 78.78	89	7b	2009	3	10.5	4,334	5.5
Hidden Valley	Bath, VA	S	38.15	– 79.76	580	6a	2007	4	2.8	1,154	5.5
Hone Quarry	Rockingham, VA	S	38.45	– 79.13	560	6a	2006/2007	7	4.9	2,019	14.0
James River State Park	Buckingham, VA	S	37.63	– 78.80	179	7a	2008	4	15.7	6,464	7.0
Jones Gap State Park	Greenville, SC	S	35.12	– 82.58	449	7b	2007/2009	6	27.9	11,495	14.0
Kentland Farm	Montgomery, VA	S	37.21	– 80.60	561	6b	2009	4	43.5	17,906	2.0
Lake Toxaway	Transylvania, NC	S	35.12	– 82.95	922	7a	2009	5	142.6	58,751	47.5
Laurel Snow	Rhea, TN	S	35.55	– 85.03	116	6b	2012	10	63.4	26,121	NA
Mountain Lake Conservancy	Giles, VA	S	37.35	– 80.53	1,192	5b	2009	10	62.8	25,874	6.0
Natural Bridge State Park	Powell, KY	S	37.77	– 83.68	336	6b	2008	10	215.8	88,905	29.8
North Creek	Botetourt, VA	S	37.54	– 79.58	354	7a	2006	10	22.8	9,394	18.1
Pickett State Park	Fentress, TN	S	36.36	– 84.48	476	6b	2012	13	13.9	5,706	NA
Pine Mountain State Park	Bell, KY	S	36.73	– 83.73	457	6b	2008	7	103.8	42,749	45.7
Pooles Creek	Rutherford, NC	S	35.41	– 82.23	403	7a	2010	5	8.0	3,284	47.0
Prentice Cooper State Forest	Marion, TN	S	35.13	– 85.42	534	7a	2007	3	11.4	4,697	7.0
Quantico	Stafford, VA	S	38.48	– 77.43	18	7a	2006/2008	10	77.7	32,017	18.6
South Mountains State Park	Burke, NC	S	35.60	– 81.63	411	7a	2007	10	98.6	40,623	45.5
Stone Mountain State Park	Wilkes, NC	S	36.39	– 81.02	536	6b	2006/2007	7	14.1	5,809	36.8
Tallulah Gorge State Park	Rabun, GA	S	34.73	– 83.39	435	7b	2005	14	129.2	53,230	50.6
Todd Lake	Augusta, VA	S	38.36	– 79.20	601	6a	2006/2007	7	50.7	20,905	13.8
Whiteside Mountain	Jackson, NC	S	35.08	– 83.13	1,441	6b	2009	1	1.5	610	0.0

[a] Range: N = Northern. S = Southern.

[b] Determined using interactive plant hardiness zone map available online: http://planthardiness.ars.usda.gov/PHZMWeb/InteractiveMap.aspx.

[c] Total number of mother trees per provenance from which seed was collected.

[d] Based on an average of 412 seeds/g (Barbour and others 2008).

[e] Based on 30-day Petri dish germination assays conducted at 22 °C, 16:8 L:D, and with two 50-seed replications per provenance.

NA = germination testing not completed at the time this article was written.

 

Figure 5. Freshly collected ripe seed cones of eastern hemlock from Minnewaska State Park, NY (left). Seeds of eastern hemlock collected from the Great Smoky Mountains National Park, packaged and ready for cold storage (right). Each packet represents seed from an individual mother tree. (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

So far, the eastern and Carolina hemlock gene conservation project has collected an estimated 2.5 million seeds. This number is impressive, but it is important to point out that only a small portion of this seed is actually usable to meet the conservation objectives outlined previously. At the end of each seed collection year, Camcore conducts provenance level seed viability tests using Petri dish germination assays conducted at 22°C (71.6 °F), 16:8 hours light:dark, and with two 50-seed replications per population. Based on these tests, average seed viability was low and highly variable for both species; averaging 20 percent (range 0 to 59 percent) and 17 percent (range 0.5 to 44 percent) for the eastern and Carolina hemlock seed reserves, respectively. These germination values are lower than expected for seed from natural stands of these species (reported at 25 to 35 percent by Godman and Lancaster 1990 and Tighe and others 2005) and can be expected to continue decreasing over time in cold storage. This condition highlights the importance of establishing both germplasm reserves in seeds banks and conservation seed orchards to ensure the long-term survival of both hemlock species.

Establishment of Seed Orchards and Reserves

Since 2008, Camcore and its associates have planted more than 2,000 hemlock seedlings into 5 hemlock seed orchards at locations inside and outside of the United States (table 3). The two Carolina hemlock plantings inside the United States are within the native range of the species in North Carolina and were established by Camcore at the North Carolina Department of Agriculture/NC State University Upper Mountain Research Station in Ashe County. Because these two orchards are within the generally infested range of HWA in the southern Appalachian region, they are being monitored regularly for HWA infestation and will be protected with insecticides when necessary.

Three conservation seed orchards were planted outside of the United States in Brazil and Chile (table 3, figure 6). This region was chosen because no native hemlock species are found in South America and chances are low that HWA would ever find its way into the plantings. The particular areas within each country where the hemlocks are planted were chosen based on the results of species habitat distribution modeling software programs using FloraMap™ (Jones and Gladkov 1999) and MaxEnt (Phillips and others 2006). These

Table 3. Location and establishment data for eastern and Carolina hemlock seed orchard conservation banks that were planted inside and outside the United States.

Species	State/department, country	Latitude (D.d)	Longitude (D.d)	Year planted	Provenances (#)	Families (#)	Seedlings (#)
Carolina hemlock	North Carolina, USA	36.41	– 81.31	2008	9	53	400
Carolina hemlock	Bio Bio, Chile	– 37.70	– 73.39	2008	9	56	1,140
Eastern hemlock	Santa Catarina, Brazil	– 26.09	– 50.26	2010	7	25	167
Carolina hemlock	Parana, Brazil	– 26.01	– 50.38	2010	9	37	182
Carolina hemlock	North Carolina, USA	36.40	– 81.32	2012	10	33	315

Figure 6. Ricardo Paim and Laercio Duda with a newly planted eastern hemlock seedling in the conservation seed orchard in Santa Catarina, Brazil (left). Saplings in the Carolina hemlock conservation seed orchard at the North Carolina Department of Agriculture /North Carolina State University Upper Mountain Research Station in Ashe County, NC (right). (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

programs use geographic coordinate and elevational data of known natural populations within the geographic range of a species to predict the most suitable climatic matches in areas outside of the species' natural range. FloraMap™ uses mean monthly precipitation, mean monthly temperature, and mean monthly diurnal temperature for all 12 months of the year (36 variables total). MaxEnt is programed to use a number of different bioclimatic variables found on the WorldClim Global Climate Database (WorldClim 2013). After the programs have determined the climatic conditions across the range of input sites (provenances), they then predict other regions of the world with similar climates where the species has a reasonable probability of occurrence/survival. In the case of eastern and Carolina hemlock, the programs indicated the areas in southern Brazil and south-central Chile where the seed orchards have been planted. The eastern and Carolina hemlock sites in Brazil are being maintained by Rigesa MeadWestvaco, and the Carolina hemlock site in Chile is managed by Arauco Bioforest. Both companies are members of the Camcore program and have donated their time, effort, and land for this gene conservation effort.

Table 4. Summary of eastern and Carolina hemlock seed submissions made to the USDA Agriculture Research Service National Center for Genetic Resource Preservation (Fort Collins, CO) via the USDA Forest Service National Seed Laboratory (Dry Branch, GA).

Species	Year submitted	Provenances (#)	Families (#)	Seeds (g)
Carolina hemlock	2003	3	Bulks	60
Carolina hemlock	2011	13	47	235
Eastern hemlock	2011	19	83	415
Carolina hemlock	2012	3	5	10
Eastern hemlock	2012	25	83	362

Camcore has also established seed reserves of both hemlock species at two germplasm repositories inside the United States (table 4). The first is at the USDA Agricultural Research Service National Center for Genetic Resource Preservation (NCGRP) located in Fort Collins, CO. In total, 1,082 g (38 oz) of seed (777 g [27 oz] and 305 g [11 oz] for eastern and Carolina hemlock, respectively) have been submitted to this facility for long-term preservation. The USDA Forest Service National Seed Laboratory in Dry Branch, GA facilitated this submission. The second repository is the Camcore seed bank at NC State University in Raleigh, NC, where all seed that has not been distributed for seed orchard establishment, submitted to the NCGRP, or used for germination testing currently resides.

Conclusions and Future Objectives

Ongoing work in hemlock genetic resource conservation is focused on two main objectives. The first is the expansion of the seed orchard program to include additional plantings of both species inside and outside the United States. Inside the country, orchards will be established both within the native range of the hemlocks (monitored and insecticide treated when needed) and on USDA Forest Service sites in the Ozark Mountains of Arkansas. With increasing restrictions on the international movement of tree seeds for research and planting, locations for plantings outside the United States are more uncertain, but plans are in place for additional orchards in Brazil and Chile.

The second objective for the ongoing work is additional seed collections. For Carolina hemlock, emphasis will be on the 11 populations in which seed has not yet been collected. Collections of eastern hemlock will focus on locations not yet well represented in seed stocks, including the high-diversity pocket in New York and southern New England and the disjunct populations in Alabama, Kentucky, and Tennessee.

Although much remains to be accomplished, the first 10 years of the Camcore/USDA Forest Service cooperative hemlock gene conservation project has been a success. It has amassed the largest genetic base of eastern and Carolina hemlock that exists outside of natural stands, established strategic seed reserves in seed banks for both species, and initiated the process of conservation seed orchard establishment. These valuable resources will be used to address a variety of research and management objectives related to HWA control, breeding of HWA-resistant genotypes, and restoring devastated hemlock ecosystems in eastern North America.

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Forest Nursery Seedling Production in the United States—Fiscal Year 2012

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Background

The U.S. Department of Agriculture (USDA), Forest Service, State and Private Forestry (S&PF) began reporting tree planting in the United States in 1952 in an annual report, *Tree Planting in the United States*. In 2000, the report was discontinued amid growing concern over the manner in which much of the tree planting data were collected.

The data for State, nonindustrial private, and industry lands were gathered and reported each year by the 50 State forestry agencies. By 2000, mergers in the forest industry and the divestiture of their timberlands shifted ownership to timber investment management organizations and real estate investment trusts. Decreased funding of State forestry agencies and reduced staffing made it increasingly difficult for them to gather the data. In addition, the tree planting data collected from some Federal agencies became difficult to gather after the 9/11 terrorist attack, when reporting restrictions were implemented by Homeland Security legislation. As a result, it was difficult to maintain confidence in the robustness of the data used for creating the *Tree Planting Report in the United States*.

In 2010, the USDA Forest Service, Forest Inventory and Analysis (FIA) program and S&PF developed a statistically valid means for reporting tree planting. The data are reported in this new *Forest Nursery Seedling Production in the United States* and were developed through the use of an empirical source and a statistical approximation.

Current Methodology

The empirical data were produced by S&PF in collaboration with Auburn University, the University of Idaho, and Purdue University. Each university was responsible for collecting forest tree seedling production data directly from the forest and conservation nurseries that grow forest tree seedlings in their region of the United States (Auburn University collected from 12 States in the Southeast, the University of Idaho collected from 17 States in the West, and Purdue University collected from 21 States in the Northeast and Midwest). The statistical approximation is derived from FIA estimates in which tree planting area is based on ground plots collected by States over 5-, 7-, or 10-year periods and compiled as an average annual estimate for the associated period. FIA estimates of acres of trees planted by State may not correlate with the estimates produced by nursery production surveys. However, total acres by region provide a reasonable comparison between the two methods. Data collected are reported by hardwood and conifer seedlings produced and acreage planted of each (table 1) and by bareroot and container seedlings produced (table 2).

The following assumptions were used in compiling this report:

1. The number of seedlings reported by the participating forest and conservation nurseries was the number of shippable seedlings produced for distribution in the 2012 planting season (i.e., seedlings to be planted from the fall of 2011 to the spring of 2012). Some species of forest seedlings require two or more growing seasons to reach accepted forest and conservation seedling size standards, so not all seedlings in production at a nursery at any given time are considered shippable (i.e., available for distribution). Therefore, only shippable seedlings were counted.

2. All of the seedling production reported in this survey met the grading standards for the respective nurseries (i.e., cull seedlings were not included in the estimates). Production estimates are often based on seedbed inventories of seedlings meeting grading standards. However, for cases in which nurseries ship seedlings by weight as opposed to examining and counting each seedling, landowners and tree planters often plant every seedling that is shipped to them, including any cull seedlings.

3. Seedling production data were collected from all the major nurseries that produced forest and conservation tree seedlings for the 2012 planting season. Considerable effort

was made to contact all producers of forest and conservation seedlings. The universities collecting the survey data reported, with few exceptions, that the major producers were included in the results.

4. All seedlings reported in this survey were produced for reforestation and conservation projects. Some of the nurseries that participated in this survey, especially in the Northern United States, produce seedlings for ornamental use, Christmas tree production, or other horticultural purposes. Private nurseries in the Northern United States were asked to report only seedling production destined for conservation and reforestation planting.

5. Forest tree seedlings remain in the general area where they are produced. Forest and conservation seedlings are routinely shipped across State borders and at times across international borders. It is assumed that, on average, the number of seedlings imported into a State is equal to the number of seedlings exported from that State. In the Lake States (Michigan, Minnesota, and Wisconsin), a significant amount of container seedlings produced in Canada are used for planting on State- and county-owned land. Estimates of the amount of seedlings shipped from Canada to the Lake States were obtained from the State nursery programs in these States. In a similar fashion, seedlings produced in forest industry nurseries in Canada are planted on industrial forest land in Maine. Estimates of the amount of Canadian-grown seedlings planted in Maine were provided by the forest industry.

6. Dividing the number of seedlings shipped by forest and conservation nurseries by the average number of stems planted per acre is an appropriate proxy of the number of acres of trees planted in the 2012 planting season. These estimations do not include direct seeding or natural forest regeneration activities. They are sufficiently robust to provide an accurate indication of tree planting over time.

7. Respondents to the production survey were asked to report only hardwood and conifer trees produced; i.e., they were asked not to include shrubs in production estimates. However, many conservation and restoration plantings include shrubs and herbaceous plants, which are used to address wildlife, biodiversity, or other management objectives. The average number of stems planted per acre used to estimate acres planted may include shrubs in some operations. Using only tree production to estimate acres planted would result in an underestimate of planted acreage where a mixed planting of shrubs and trees occurred. For example, in the Northern United States, State-owned nurseries produced more than 4 million shrubs in addition to the more than 54 million trees reported for the 2012 planting season.

Table 1. Hardwood and conifer tree seedling production and acres planted for each State and each region during the 2011-to-2012 planting year.

State	Hardwood seedlings produced	Hardwood acres planted[a]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[a]	Total seedlings produced	Total acres planted[a]	FIA Data acres planted[j]
Southeast								
Florida[b]	4,240,000	7,709	31,502,000	—	57,276	35,742,000	64,985	140,247
Georgia[b]	7,207,000	13,104	296,090,000	—	538,345	303,297,000	551,449	196,602
North Carolina[b]	—	—	63,223,000	—	114,951	63,223,000	114,951	108,286
South Carolina[b]	3,540,000	6,436	105,822,000	—	192,404	109,362,000	198,840	55,479
Virginia[b]	805,000	1,464	25,065,000	—	45,573	25,870,000	47,036	92,707
Regional totals	15,792,000	28,713	521,702,000	0	948,549	537,494,000	977,262	593,320
South Central								
Alabama[b]	899,000	1,635	87,308,000	—	158,741.82	88,207,000	160,376	263,720
Arizona[b]	12,686,000	23,065	86,940,000	—	158,073	99,626,000	181,138	156,973
Kentucky[c]	1,865,512	4,289	308,030	—	708	2,173,542	4,997	1,479
Louisiana[b]	4,580,000	8,327	26,409,000	—	48,016	30,989,000	56,344	166,984
Mississippi[b]	1,038,000	1,887	87,102,000	—	158,367	88,140,000	160,255	192,746
Oklahoma[b]	1,044,000	1,898	3,196,000	—	5,811	4,240,000	7,709	25,434
Tennessee[b]	1,508,000	2,742	5,634,000	—	10,244	7,142,000	12,985	22,489
Texas[b]	37,000	67	78,870,000	—	143,400	78,907,000	143,467	113,125
Regional totals	23,657,512	43,911	375,767,030	0	683,361	399,424,542	727,272	942,949
Northeast								
Connecticut	—	—	—	—	—	—	—	—
Delaware	—	—	—	—	—	—	—	—
Massachusetts	—	—	—	—	—	—	—	—
Maryland[c]	897,180	1,631	1,166,800	—	2,121	2,063,980	3,753	—
Maine[d, i]	—	—	0	2,000,000	2,500	2,000,000	2,500	8,284
New Hampshire[c]	26,500	48	179,075	—	326	205,575	374	—

Table 1. Hardwood and conifer tree seedling production and acres planted for each State and each region during the 2011-to-2012 planting year (continued).

State	Hardwood seedlings produced	Hardwood acres planted[a]	Conifer seedlings produced	Canadian conifer imports	Conifer acres planted[a]	Total seedlings produced	Total acres planted[a]	FIA Data acres planted[j]
Northeast continued								
New Jersey[c]	1,239,540	2,254	429,950	—	782	1,669,490	3,035	—
New York[e]	108,322	120	810,544	—	901	918,866	1,021	203
Pennsylvania[c]	1,148,777	2,089	3,174,903	—	5,773	4,323,680	7,861	1,391
Rhode Island	—	—	—	—	—	—	—	—
Vermont	—	—	—	—	—	—	—	—
West Virginia[c]	764,500	1,390	81,000	—	147	845,500	1,537	—
Regional Totals	4,184,819	7,532	5,842,272	2,000,000	12,549	12,027,091	20,081	9,878
North Central								
Iowa[e]	1,447,450	2,412	410,000	—	683	1,857,450	3,096	—
Illinois[c]	1,452,705	3,340	308,510	—	709	1,761,215	4,049	5,062
Indiana[d]	3,774,099	5,806	1,417,592	—	2,181	5,191,691	7,987	1,331
Michigan[b, i]	5,697,300	10,359	13,731,000	1,500,000	15,231	20,928,300	25,590	11,899
Minnesota[b, i]	1,684,775	3,063	13,239,500	4,275,000	29,191	19,199,275	32,254	20,059
Missouri[c]	4,095,900	9,416	766,375	—	1,762	4,862,275	11,178	—
Ohio[c]	5,000	11	—	—	—	5,000	11	3,775
Wisconsin[f, i]	1,964,850	2,456	13,875,400	1,000,000	18,594	15,840,250	21,050	9,413
Regional Totals	20,122,079	36,864	43,748,377	6,775,000	68,351	69,645,456	105,214	51,540
Great Plains								
Kansas[b]	100,000	182	—	—	—	100,000	182	—
North Dakota[b]	1,720,000	3,127	1,500,000	—	2,727	3,220,000	5,855	—
Nebraska[b]	800,000	1,455	1,200,000	—	2,182	2,000,000	3,636	—
South Dakota[b]	800,000	1,455	419,000	—	762	1,219,000	2,216	—
Regional Totals	3,420,000	6,218	3,119,000	0	5,671	6,539,000	11,889	0
Intermountain								
Arizona[b]	38,060	69	1,520	—	3	39,580	72	—
Colorado[b]	750,000	1,364	750,000	—	1,364	1,500,000	2,727	—
Idaho[b]	1,002,750	1,823	4,667,500	—	8,486	5,670,250	10,310	4,287
Montana[b]	344,000	625	525,000	—	955	869,000	1,580	5,142
New Mexico[b]	28,400	52	72,500	—	132	100,900	183	—
Nevada[b]	1,000	2	500	—	1	1,500	3	—
Utah	—	—	—	—	—	—	—	—
Wyoming	—	—	—	—	—	—	—	—
Regional Totals	2,164,210	3,935	6,017,020	0	10,941	8,181,230	14,875	9,429
Alaska								
Alaska	—	—	—	—	—	—	—	806
Pacific Northwest								
Oregon[g]	2,529,500	7,227	58,359,500	—	166,741	60,889,000	173,969	88,379
Washington[g]	2,719,800	7,771	78,323,000	—	223,780	81,042,800	231,551	54,179
Regional Totals	5,249,300	14,998	136,682,500	0	390,521	141,931,800	405,520	142,558
Pacific Southwest								
California[h]	16,400	30	14,202,400	—	25,823	14,218,800	25,852	29,535
Hawaii[h]	100,000	182	5,000	—	9	105,000	191	—
Regional Totals	116,400	212	14,207,400	0	25,832	14,323,800	26,043	29,535
Totals	**74,706,320**	**142,382**	**1,107,085,599**	**8,775,000**	**2,145,775**	**1,190,552,819**	**2,288,156**	**1,780,014**

[a] Acres planted were estimated assuming:
[b] 550 stems/acre
[c] 435 stems/acre
[d] 650 stems/acre
[e] 600 stems/acre
[f] 800 stems/acre
[g] 350 stems/acre
[h] 450 stems/acre
[i] Totals include an estimate of conifers produced in Canada for distribution to neighboring states; bareroot imports for ME and container for other states.
[j] Average annual acreage planted was estimated for all States on 5-year cycles except Alabama, Louisiana, and North Carolina are 7-year cycles and Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, and Washington are 10-year cycles; data generated by R. Harper.

Table 2. Bareroot and container tree seedling production for each State and each region during the 2011-to-2012 planting year.

State	Bareroot	Container	Total seedlings produced
Southeast			
Florida	28,598,000	7,144,000	35,742,000
Georgia	162,966,000	140,331,000	303,297,000
North Carolina	45,709,000	17,514,000	63,223,000
South Carolina	107,704,000	1,658,000	109,362,000
Virginia	25,870,000	—	25,870,000
Regional totals	370,847,000	166,647,000	537,494,000
South Central			
Alabama	82,048,000	6,159,000	88,207,000
Arizona	99,626,000	—	99,626,000
Kentucky	2,173,542	—	2,173,542
Louisiana	30,364,000	625,000	30,989,000
Mississippi	80,111,000	8,029,000	88,140,000
Oklahoma	4,195,000	45,000	4,240,000
Tennessee	7,142,000	—	7,142,000
Texas	78,907,000	—	78,907,000
Regional totals	384,566,542	14,858,000	399,424,542
Notheast			
Connecticut	—	—	—
Delaware	—	—	—
Massachusetts	—	—	—
Maryland	2,063,980	—	2,063,980
Maine	—	—	2,000,000
New Hampshire	205,575	—	205,575
New Jersey	544,490	1,125,000	1,669,490
New York	854,400	64,466	918,866
Pennsylvania	4,314,580	9,100	4,323,680
Rhode Island	—	—	—
Vermont	—	—	—
West Virginia	845,500	—	845,500
Regional totals	8,828,525	1,198,566	12,027,091
North Central			
Iowa	1,842,950	14,500	1,857,450
Illinois	1,759,800	1,415	1,761,215
Indiana	5,011,691	180,000	5,191,691
North Central continued			
Michigan	19,428,300	—	20,928,300
Minnesota	9,364,275	5,560,000	19,199,275
Missouri	4,454,775	407,500	4,862,275
Ohio	—	5,000	5,000
Wisconsin	15,840,100	150	15,840,250
Regional totals	57,701,891	6,168,565	69,645,456
Great Plains			
Kansas	—	100,000	100,000
North Dakota	3,125,000	95,000	3,220,000
Nebraska	1,125,000	875,000	2,000,000
South Dakota	1,180,000	39,000	1,219,000
Regional totals	5,430,000	1,109,000	6,539,000
Intermountain			
Arizona	—	39,580	39,580
Colorado	—	1,500,000	1,500,000
Idaho	3,000,000	2,670,250	5,670,250
Montana	300,000	569,000	869,000
New Mexico	100	100,800	100,900
Nevada	1,500	—	1,500
Utah	—	—	—
Wyoming	—	—	—
Regional totals	3,301,600	4,879,630	8,181,230
Alaska			
Alaska	—	—	—
Pacific Northwest			
Oregon	34,290,000	26,599,000	60,889,000
Washington	51,600,000	29,442,800	81,042,800
Regional totals	85,890,000	56,041,800	141,931,800
Pacific Southwest			
California	—	14,218,800	14,218,800
Hawaii	—	105,000	105,000
Regional totals	—	14,323,800	14,323,800
Totals	**918,565,558**	**272,001,361**	**1,190,552,819**

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Entire book:	Pallardy, S.G. 2008. Physiology of woody plants. 3rd ed. Burlington, MA: Academic Press. 454 p.
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